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Studies of lipid peroxidation mechanisms that lead to the development of warmed-over flavour in red meat products

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Background

Current trends show that meat consumers are showing an increased demand for pre-cooked "heat and eat" convenience meals. The worldwide market potential for meat-based ready meals was estimated to be approximately $20 billion in 2000 with an annual growth of 4.0%.

Home meal replacement (HMR) is one of the fastest growing segments of the food industry. Meat usually represents the main component of a meal and the form in which it is presented has an important impact on consumer acceptance. For example, over 30% of beef produced goes into cured meat products and while these products are oxidatively stable, they do not taste like freshly cooked beef and are not acceptable to consumers as a HMR. Consumers display a clear preference for the flavour and aroma of freshly cooked beef and expect these to be present in a beef-based HMR.

Once cooked, beef is usually served immediately and not kept stored for later consumption. This is due to the rapid development of the specific and undesirable flavour associated with reheating refrigerated cooked beef. In 1958, the American meat researchers Margaret Tims and Betty Watts introduced the term "warmed-over" to describe this off flavour and in the 45 years that have followed much research effort has been devoted to its solution.

Warmed-over flavour (WOF) results from the rapid oxidation of lipids in cooked meat. Chilled raw meat is oxidatively stable for several weeks whereas in the cooked product rancidity can be detected within hours. WOF is characterised by loss of fresh meat aroma and the development of flavours and aromas described by consumers as "stale", "rancid", "painty" or "cardboard-like". WOF is a major problem in the restaurant, fast-food, institutional-service and airline food catering industries. Therefore, the production of novel heat and serve pre-cooked beef meals for these markets and the HRM market must address the issue of WOF development during storage and subsequent serving of the product.

The early development of WOF in ready-to-eat meat meals could be masked through the inclusion of condiments and sauces. However, free radicals, lipid hydroperoxides and malondialdehyde produced during the oxidation process have been implicated in the pathophysiology of diseases such as atherosclerosis and cancer. The prevention of WOF in pre-cooked meat products has potentially important health implications as well as its impact on consumer acceptability.

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Scope

This project had two major objectives. Firstly, we were interested in progressing the strategic science of lipid oxidation of red meat. It had been known since the late 1970's that WOF didn't originate from the depot fat of adipose tissue in meat. The American workers who made this observation proposed that WOF resulted from the oxidation of polyunsaturated fatty acids found in phospholipids, the lipids that make up the membranous structures of the muscle cell.

Not much research has focussed on the composition and structure of muscle phospholipids to date and even less on bovine muscle phospholipids. Muscle tissue is rich in a special class of phospholipids known as plasmalogens. In most phospholipids both the long chain fatty acids are attached to the glycerol moiety by ester linkages but in plasmalogens one of the fatty acids is linked to glycerol by a vinyl ether bond. This unique chemical structure of plasmalogens is postulated to protect it's long chain unsaturated fatty acids from oxidation.

Current models of lipid oxidation in food systems are based on ester-linked fatty acids and are therefore of limited value to meat where approximately 50% of the total phospholipids are plasmalogens. In this project, we therefore not only examined phospholipid composition of bovine membranes but also their oxidative susceptibility.

Our second objective was to investigate processing methods or treatments aimed at reducing or eliminating WOF in chilled pre-cooked beef. Two generic approaches to this problem immediately came to mind. Firstly, antioxidants can be added to the meat products. If this approach is taken then one can decide between synthetic or natural compounds. Consumers generally view addition of synthetic or "artificial" compounds as unfavourable whereas "natural" compounds are associated with good health. Most natural antioxidant compounds are plant-derived polyphenols. Rich sources of these are found in extracts from green tea, grapes and olives. While the antioxidant efficacy of these extracts, when added to approximately 1% levels in meat products, is well established we had several concerns regarding their use. At these levels the extracts can impact adversely on meat flavour but more importantly polyphenols are potent chelators of iron thereby potentially reducing its bioavailability from cooked meat.

Alternatively meat products can be cooked in vacuum pouches, chilled and then reheated prior to serving. This is the so called "sous vide" (with vacuum) technique. Sous vide meat products are generally cooked at temperatures of 70-90°C for sufficient times to ensure pasteurisation. Sous vide cooked meat can be stored for 10 days prior to the development of detectable levels of WOF. A major drawback of the low cooking temperature in the sous vide

process is lack of desirable meat aromas, normally produced by browning during oven roasting.

In view of the potential problems associated with natural antioxidants alluded to above and the well established nature of the sous vide process, we chose to attempt to develop novel processing and treatments for the prevention of WOF in precooked meat products.

1. Sensory and chemical evaluation of warmed-over flavour in cooked beef.

Past research by other workers into warmed-over flavour in beef tended to focus either on sensory evaluation of aroma and flavour, or the chemically determined products of lipid oxidation, but few looked at both. Fewer still examined the loss of individual fatty acids to see if their loss correlated firstly with increased levels of chemically determined oxidation products and secondly with the sensory results.

The objective of this part of the project was to address, what we believed to be, shortcomings in past work in this field. For the first time, we hoped to obtain comprehensive data regarding the relationship between the oxidation of fatty acids and the relationship of this to the sensory perception of warmed-over aroma and flavour.

Our research showed that:

- Taste panellists found that 80% of maximum warmed-over aroma was evident in reheated beef slices (blade and loin) within 24 hours refrigerated storage and maximum levels were reached after two days refrigerated storage.

- The taste panel reported similar trends for warmed-over flavour to that of warmed-over aroma in reheated blade and loin slices but the panel gave higher scores for warmed-over flavour to blade slices compared to scores given for loin slices.

- The taste panel reported no changes in tenderness or juiciness in reheated beef slices that had been in refrigerated storage for up to six days, so the observed decrease in quality rating of reheated beef following refrigerated storage was due solely to the development of warmed-over aromas and flavours.

- Chemical analysis of the sensory evaluated samples showed that blade had a higher total lipid, neutral lipid and phospholipid content than loin.

- Chemically determined lipid rancidity, as thiobarbituric acid reactive substances (TBARS), in sensory evaluated beef slices was found to increase during refrigerated storage and tended to be higher in blade compared to loin slices.

- Fatty acid analysis of the sensory evaluated beef samples indicated no loss of saturated, monounsaturated, or diunsaturated fatty acids from the neutral or phospholipid fractions of the cooked beef.

- Total phospholipid polyunsaturated fatty acids showed a dramatic decline with increasing time of refrigerated storage in both blade and loin slices. While both the blade and loin samples displayed similar rates of phospholipid polyunsaturated fatty acid decline, blade samples always displayed higher levels of phospholipid poly-unsaturated acids.

- Four of the six major phospholipid polyunsaturated fatty acids in the cooked beef samples were shown to account for the observed decline of total poly-unsaturated fatty acids during refrigerated storage. Each of the four polyunsaturated fatty acids contained 4 or more double bonds with arachidonic acid (20:4) being the most abundant fatty acid.

- Lipid rancidity in the sensory evaluated beef samples correlated strongly with loss of phospholipid polyunsaturated fatty acids thus establishing that oxidation of these fatty acids was responsible for the increasing TBARS values observed during increasing time of refrigerated storage.

- Warmed-over aroma and flavour sensory scores correlated highly with TBARS value. The TBARS value for half maximal sensory warmed-over aroma and flavour showed that detectable off-flavours and aromas developed within five hours of refrigerated storage of cooked beef.

2. Characterisation of muscle phospholipid composition

Having established that the bulk of warmed-over flavour and aroma arises from the oxidation of a single polyunsaturated fatty acid, i.e. arachidonic acid, investigations then focused on where and in what form the arachidonic acid occurred within the muscle cell structure.

Since it was well established that polyunsaturated fatty acids such as arachidonic acid are found predominantly in membranes surrounding intracellular organelles such as mitochondria and endoplasmic reticulum these intracellular structures were isolated from muscle cells for extensive lipid characterisation. In muscle cells the endoplasmic reticulum has become

highly specialised in uptake and release of calcium ions during contraction and relaxation activity and is called the sarcoplasmic reticulum.

In biological membranes the polyunsaturated fatty acids that are chemically bound to glycerol and phosphate are known as phospholipids. Depending on the nature of the phosphate group, the phospholipids are assigned to four major classes. Within each phospholipid class the attachment of fatty acids to the glycerol portion of the molecule can occur in different positions giving rise to different molecular species within the same class, adding a further layer of complexity to the structure of these compounds.

Our research showed that:

- Of the two major muscle membrane systems, mitochondria and sarcoplasmic reticulum, there was approximately twice as much mitochondria as sarcoplasmic reticulum in the three muscles studied in this work.

- In each of the three muscles, mitochondria contained higher levels of total phospholipids than sarcoplasmic reticulum membranes.

- In both membranes from the three muscles the four phospholipid classes, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine plus phosphatidylinositol (PI/PS) accounted for approximately 91% of the total phospholipids. PC was the major phospholipid class accounting for approximately 60% of total phospholipids, followed by PE (23%) and PI/PS (8%).

- While PC was the most abundant phospholipid class it contained the lowest level of polyunsaturated fatty acids (15% of total). PE had the highest levels of polyunsaturated fatty acids (50%) and PI/PS was intermediate with 25%.

- In both membranes in the three muscles examined, the most abundant polyunsaturated fatty acid was arachidonate (20:4) and oxidation of this fatty acid had previously been shown to lead to development of warmed-over flavour.

- Arachidonate levels were highest in the PE phospholipid class in both membranes but levels were four times higher in mitochondria compared to sarcoplasmic reticulum membranes. Therefore oxidation of mitochondrial PE arachidonate is the major source of warmed-over flavour compounds in bovine muscle.

- Total muscle arachidonate was higher in forequarter muscle (blade) than in primal hindquarter tenderloin suggesting that there would be more potential for warmed-over flavour problems in convenience meals prepared from these cuts compared to primals**.**

3. Oxidative susceptibility of phospholipids within the major muscle membrane systems.

Our research established that oxidation of the polyunsaturated fatty acid, arachidonic acid, was mainly responsible for the rapid development of warmed-over flavour in chill stored cooked beef. Furthermore, mitochondria were shown to be the major reservoir of arachidonic acid in muscle.

However, this did not prove that oxidation of mitochondrial arachidonic acid was responsible for development of warmed-over flavour. Other researchers had shown that susceptibility of fatty acid oxidation depended not only on the class of phospholipid but also the molecular species involved.

Therefore, we undertook an investigation that looked at the oxidative susceptibility of arachidonic acid in the phospholipids of mitochondria and sarcoplasmic reticulum. We hoped that such an investigation would lead to clearer insights into the mechanisms of oxidation that eventually result in the development of unacceptable aromas and flavours in beef.

Our research showed that:

- Muscle lipids of mitochondrial membranes (Mito) are more susceptible to oxidation than their counterparts associated with the sarcoplasmic reticulum (SR).

- The rate of Mito lipid oxidation was determined to be 50% faster than that observed for SR.

- Up to 80% reduction of membrane arachidonic acid occurred in one hour following initiation of lipid oxidation.

- It is estimated that in Mito, 40% of arachidonic acid is involved in phospholipid species containing a saturated fatty acid at the 1-position and arachidonic acid at the 2-position of the glycerol backbone both of which are ester-linked (diester form). The distribution of diester arachidonic acid phospholipid species was the same in two forequarter beef cuts as that found in beef tenderloin.

- SR contained less diester arachidonic acid phospholipid species than Mito but displayed a higher content of species in which the saturated fatty acid at position-1 and arachidonic acid at postion-2 of the glycerol backbone were vinyl ether and ester linked, respectively (plasmalogen form).

- SR plasmalogen content varied between beef cuts, being lower in forequarter cuts of blade and shin (muscles *Triceps brachii* and *Biceps brachii*, respectively) compared to primal tenderloin cut (muscle *Longissmus dorsi*).

- Polyunsaturated fatty acids in plasmalogen phospholipid species have been reported to be less susceptible to oxidation than their diester counterparts. Therefore, we propose that the greater oxidative susceptibility of Mito arachidonic acid compared to that in SR could be explained on the basis of its higher levels of diester arachidonic acid phospholipid species.

- The lower levels of plasmalogen phospholipid species in forequarter cuts to that seen in tenderloin could indicate more potential for warmed-over flavour problems in these beef cuts.

4. Ribose treatment of beef: An examination of processing, cooking and storage conditions and their impact on warmed-over flavour.

This project was concerned with the strategic science of lipid oxidation leading to the development of warmed-over flavour in beef. The results obtained thus far were consistent with this objective.

The final objective of the project was to investigate practical methods of preventing or reducing the development of warmed-over flavour in chill stored cooked beef and beef products. The objective scope was taken to mean that any process or treatment resulting from the investigation should lend itself to uptake by industry.

Furthermore, we wanted to examine novel ways to solve the problem. For this reason we chose not to investigate vacuum or modified atmosphere packaging for preventing warmed-over flavour in cooked beef products. For the same reason we were not keen to add to the plethora of "natural" and synthetic antioxidants that had already been extensively researched.

We pursued the idea of attenuating production of antioxidant compounds that are produced naturally during the cooking of beef. These compounds are part of the complex series of reactions that are involved in the production of beefy aromas and flavours. The generic name of these reactions is the Maillard

reaction which is also responsible for the development of the unique flavour and aroma characteristics in coffee and chocolate.

Antioxidant intermediates produced by the Maillard reaction during the cooking of beef begin with the reaction between naturally occurring sugars and muscle proteins. Therefore we chose to attempt to attenuate this reaction by addition of those sugars that occur naturally in beef. To this end, we investigated the addition of glucose, fructose and ribose to beef cuts prior to cooking as a means of producing natural antioxidants that could potentially reduce the development of warmed-over aroma and flavour during chilled storage of cooked beef

Our research showed that:

- Of the three sugars investigated, ribose was the only one that effectively reduced lipid oxidation in the cooked product during chilled storage.
- Antioxidant efficacy was demonstrated to relate to internal meat temperature during cooking rather than browning of the meat surface.
- The antioxidant efficacy in the ribose-treated beef increased as the internal meat temperature increased from 70°C to 90°C.
- A sensory evaluation of ribose-treated beef, cooked to an internal temperature of 85°C, was undertaken using a taste panel familiar with warmed-over beef flavour.
- The panellists gave significantly higher scores for meat flavour and aroma in the ribose treated meat.
- Conversely, the panellists scored the ribose-treated beef lower in warmed-over flavour and aroma than the untreated product.
- Sensory scores for tenderness did not differ significantly between control and ribose-treated beef.
- The panellists scored the ribose-treated beef as more juicy than the untreated beef.
- The relationship between chemically determined lipid oxidation (TBARS value) and sensory scores was examined and a predictive model of "warmed-over" shelf life during chilled storage was developed.

- Warmed-over shelf life was defined, as the time in hours that cooked beef could be stored chilled prior to the development of sufficient lipid oxidation that would be detected by consumers following reheating of the chilled product.

- For untreated beef cooked to internal temperatures of 75°C, 80°C, 85°C or 90°C the predicted warmed-over shelf life was 5.3, 5.5, 7.5, and 9.5 hours, respectively.

- The corresponding shelf life for the ribose-treated product, at the same temperatures, was 5.6, 8.1, 15.7, and 25.3 hours.

- Therefore, in ribose-treated meat, the warmed-over shelf was extended by 0.3, 2.6, 8.2, and 15.8 hours for beef cooked to internal temperatures of 75°C, 80°C, 85°C and 90°C, respectively.

5. Potential Applications for Ribose-treated beef products.

Ribose has the potential to be a useful ingredient in pre-cooked ready to eat beef products due to its dual functionality of antioxidant and flavour enhancer. Ribose could be introduced into beef carcases through postexsangiunation vascular infusion and the cost effectiveness of such a process was examined.

Two types of cooked beef product were identified as potentially benefiting from ribose addition. These were emulsion cured beef and fresh beef products using cook-in-casing for roasts or sous vide for beef portions.

The emerging technology of ultra high pressure (UHP) processing was seen as a means of attenuating beneficial effects of ribose-treated beef in these type of products.

Our market research showed that:

- Ribose could be introduced into beef muscles via postexsanguination vascular infusion using the technology developed by MPSC Inc.

- The large volumes of solution required for vascular infusion of beef carcases that would be required to produce effective ribose levels are estimated to cost between $25-$100 per carcass and such costs are clearly prohibitive.

- Ribose could be used to improve colour and also reduce excess levels of nitrite in emulsion cured beef products.

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- Ribose could be used to improve flavour and oxidative stability in emulsion cured beef products.

- Use of ribose in emulsion cured beef products is estimated to increase the product cost by 2-3% per kilogram.

- Ribose addition to raw beef prior to cook-in-casing for beef roasts or sous vide cooking for beef portions is seen as a simple and effective means to obtain flavour enhancement and extended flavour shelf life in these type of products.

- The high value added nature of cook-in-casing and sous vide products should readily absorb the estimated 4-5% increase in cost per kilogram of product due to ribose addition, provided flavour and shelf life benefits are seen using current temperature and cooking times in processing these product types.

- Ultra high pressure (UHP) processing at 60°C of ribose treated beef is seen as a means of attenuating the beneficial effects of ribose above those seen using conventional technologies.

- UHP processing of ribose treated beef will have the additional benefits of improved tenderness and microbiological safety.

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Recommendations

- **Use of ribose in cooked meat products to prevent warmed-over flavour, as described in this report, is novel and therefore patentable. It is recommended that consideration be given to taking out a provisional patent on the use of ribose in warmed-over flavour prevention. If a provisional patent is taken out it is further recommended that, while it is in effect, the market potential for use of ribose in warmed-over flavour prevention be assessed. From the results of the market survey a decision can be made as to whether complete patent status is warranted.**

- **Apart from the use of ribose in the prevention of warmed-over flavour none of the findings in this report have commercial application. They do however represent novel and significant scientific findings. It is therefore recommended that the MLA give permission to the authors of this work to publish the non-commercially sensitive material in the scientific literature.**

- **Ultra high pressure (UHP) is an emerging technology with demonstrated efficacy in improvement of meat tenderisation and microbial safety. The scientific literature indicates that UHP can increase the Maillard reaction rates which we believe are similar to the beneficial reaction of ribose in cooked meat. If UHP plus mild heat can improve on the antioxidant effects of ribose seen when meat is cooked to 90°C, then warmed-over flavour would no longer be a problem. It is therefore recommended that the MLA support research into UHP processing as a means of producing meat-based shelf stable pre-cooked "heat and eat" convenience meals.**

- **Ribose occurs naturally in beef and is known to have a key role in the development of beef flavours and aromas. In this project we have shown, through sensory trials, that beef aroma and flavour is enhanced when ribose is added to pre-cooked beef above background levels. The corollary of this suggests that the higher the background levels of ribose the better the flavour of the beef. It is therefore recommended that the MLA support research into improving beef flavour by increasing the natural ribose content in beef muscles by nutritional means.**

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1. Sensory and chemical evaluation of warmed-over flavour in cooked beef.

1.1. Background

Intramuscular lipids play a key role in the important meat quality trait of flavour. The lipid components of bovine meat contribute not only to positive beef flavour but also to its deterioration. Lipid breakdown commences after slaughter and involves their oxidation.

The lipids of muscle consist of triacylglycerols (TAGS) and membrane phospholipids (PL). TAG content of muscle can vary from 0.2% to greater than 5% while the PL content remains relatively constant between 0.5% and 1.0% of wet muscle weight. Deterioration of beef flavour due to oxidation of muscle lipids has been shown to be mainly associated with the muscle PL rather than the TAGS and this is attributed to the higher content of polyunsaturated fatty acids (PUFAs) of PL compared to TAGS.

The term warmed-over flavour (WOF) was first introduced by the American meat researchers Margaret Tims and Betty Watts in 1958 to describe the rapid onset of rancidity in cooked meat during refrigerated storage. While chilled raw meat is oxidatively stable for several weeks, in cooked products rancidity can be detected within hours. WOF is described by consumers as "stale", "rancid", "painty", "cardboard-like" and is characterised by loss of fresh meat aroma with an increase in "cardboard-like" aroma. Within 1-3 days, this off-aroma is gradually replaced by a rancid "painty" aroma followed by the development of a sour taste. Consumer rejection of refrigerated cooked meat can occur in 24-48 hours storage.

At present it is known that 80% WOF compounds originate from oxidation of PUFAs in muscle PL even though the PL comprise only 1% of total muscle lipid. However, when it is taken into consideration that approximately 30% of the PL fatty acids are polyunsaturated compared to 2% of the TAGS fatty acids, it is not surprising that muscle membrane PL is the major lipid source for WOF development.

The major membrane systems in bovine muscle are the cell plasma membrane (sarcolemma), and the membranes of the internal organelles the mitochondria (Mito) and the sarcoplasmic reticulum (SR). At present it is not known which of these muscle membrane systems are more important in the development of WOF. Slow fibre type muscles contain more mitochondria and less sarcoplasmic reticulum than fast muscles. Since slow fibre type muscles tend to predominate the forequarter of beef carcases and these lower value meat cuts would be used in pre-cooked "warm and eat" convenience meals then a WOF problem may occur in forequarter muscles if mitochondrial PL are more prone to lipid oxidation than sarcoplasmic reticulum PL lipids.

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1.2 Sensory Evaluation of Warmed-over Flavour

Methodology
A series of sensory studies was conducted to determine the effects of refrigerated storage time on the development of warmed-over flavour and aroma in reheated cooked beef cuts. The cuts employed in this study were primal loin (*Longissimus dorsi* muscle*)* and forequarter blade (*Triceps brachii* muscle). The rationale for the choice of these two cuts is that high value pre-cooked "warm and eat" convenience beef meals would generally be prepared from lower value beef forequarter cuts and these cuts are known to be biochemically different from the primal cuts.

Six full beef cuts were obtained from a Meat Standards Australia (MSA) accredited butcher. Three whole blades and three whole loins, all MSA Grade 3, Cryovac packaged and aged 5 days prior to release, were stored overnight at 2°C prior to processing.

The whole cuts were trimmed of fat and connective tissue and cut into portions. The samples were trimmed to a standard size roast (approximately 300 grams) and randomly allocated to treatments (frozen storage or chilled storage). The roasts were cooked in a fan-forced oven set at 200°C to medium level doneness (final internal temperature of 75°C). The cooked roasts were rested for 10 minutes, while wrapped in aluminium foil, before being sliced into 2 mm slices. Samples allocated to frozen storage were vacuum packed in Cryovac bags and immediately frozen. The samples to be stored chilled were packed in "zip-lock" polyethylene bags and stored at 4°C until required for sensory evaluation.

For sensory evaluation, the beef slices were reheated in a temperature controlled water bath set at 75°C for 10 minutes and kept at 65°C until required for tasting, usually within 15 minutes. Each panellist was presented with 1 slice per treatment and asked to assess differences in the meat aroma, warmed-over aroma, meat flavour, warmed-over flavour, tenderness, juiciness and overall quality attributes.

At each session four (4) treatments were assessed over four storage timepoints (1, 2, 3, and 6 days):

1. Blade stored frozen
2. Loin stored frozen
3. Blade stored chilled at 4°C
4. Loin stored chilled at 4°C

Three replicate sessions were conducted for each time point. Twelve panellists were selected on their ability to discriminate small differences in meat flavour and were familiar with warmed-over flavour development in meats from previous studies. Two preliminary sessions were conducted prior to evaluation of the experimental samples and all panellists correctly selected the 3-day warmed-over treatment when compared to a frozen reference for both loin and blade cuts.

All sensory assessments were carried out under red lighting conditions in individual booths to disguise any differences due to colour or appearance. Each sample was presented on a white plate in random order with a 3 digit code to identify each sample. Panellists assessed the seven attributes using a 9-point category scale as shown in Table 1.1.

Table 1.1. Sensory profile for beef warmed-over flavour evaluation.

No.	Sensory Attribute	End Labels
1	Meat Aroma	None (1) – Very Strong (9)
2	Warmed-over Aroma	None (1) – Very Strong (9)
3	Meat Flavour	None (1) – Very Strong (9)
4	Warmed-over Flavour	None (1) – Very Strong (9)
5	Tenderness	Very Tough (1) – Very Tender (9)
6	Juiciness	Very Dry (1) – Very Juicy (9)
7	Overall Quality	Very Poor (1) – Very Good (9)

Results of sensory evaluation

The average scores for the seven sensory attributes given to reheated cooked blade and loin slices that had been previously stored refrigerated for up to 6 days are displayed graphically in Figure 1.1.

The graph labelled "Aroma" in Fig 1.1 shows the development of warmed-over aroma (WOA) and loss of meat aroma during chilled storage of cooked beef. The increase in WOA mirrored the loss of meat aroma. For aroma, there were no significant muscle differences but the increase in WOA and the decline of meat aroma with storage time was highly significant. Maximal WOA was evident following 2 days chilled storage and 40% of maximal WOA had developed during the first 24 hours of storage.

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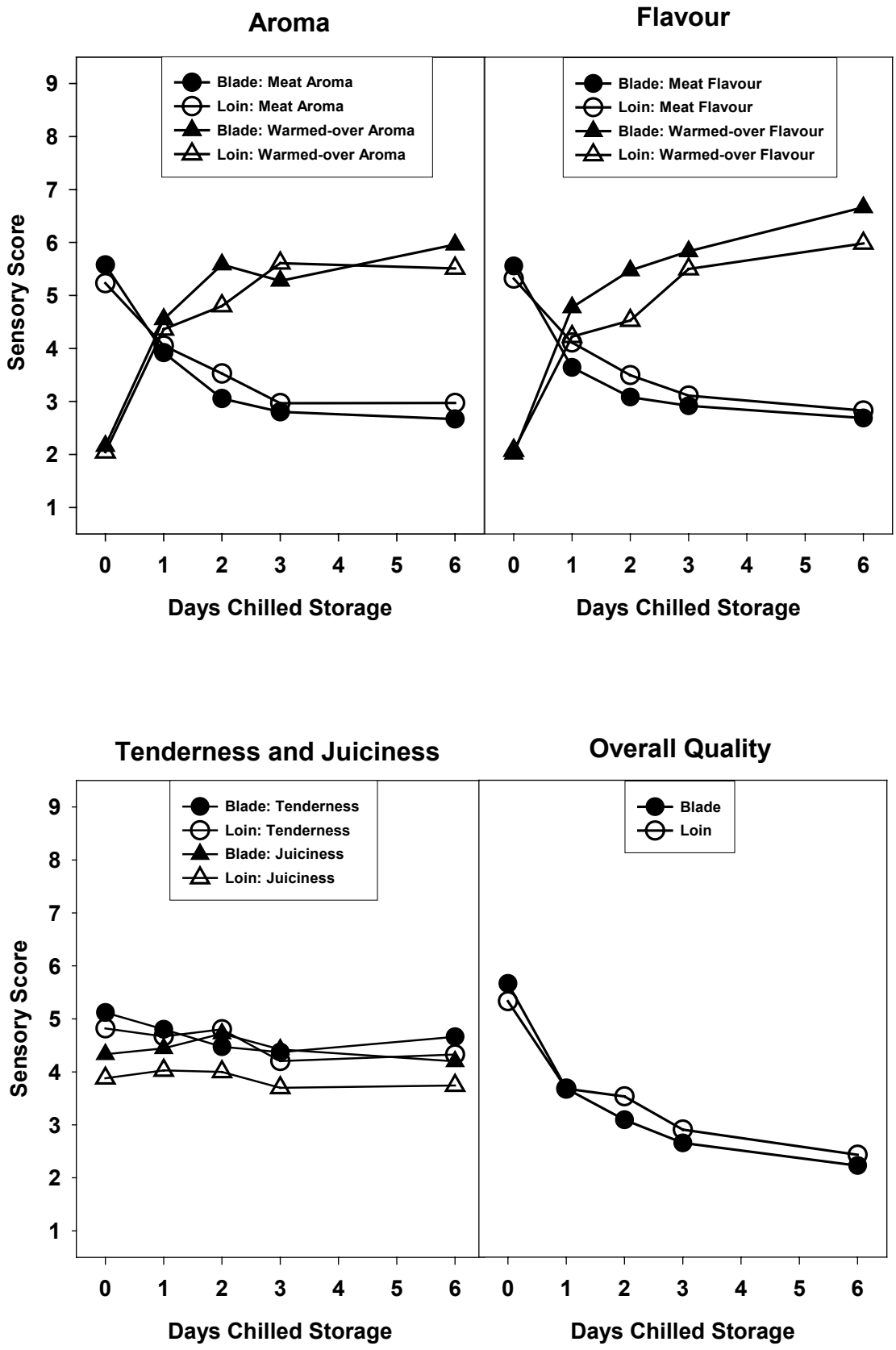

Figure 1.1. Sensory scores for aroma, flavour, tenderness, juiciness and overall quality in reheated cooked beef slices during chilled storage.

Interestingly while WOA scores did not differ between the muscles, warmed-over flavour (WOF) was consistently higher in blade samples compared to loin ("Flavour" in Fig. 1.1). Also WOF appeared to continue to increase with increasing time of chilled storage compared to WOA which appeared to plateau following 2 days storage. In the frozen control (0 day chilled storage) blade displayed somewhat higher meat flavour than 0 day loin but following 1 to 6 days chilled storage the blade consistently displayed lower meat flavour than the corresponding chilled loin samples.

Both the tenderness and juiciness sensory scores remained relatively constant throughout the storage trial with blade displaying higher juiciness than loin. Therefore, these attributes would have had little influence on the overall quality rating. The overall quality scores closely matched those observed for meat aroma and flavour loss while mirroring the increase in WOA and WOF. While both muscles scored a significant quality reduction following 24 hours chilled storage, the reduction in quality scores did not differ significantly between the muscles.

1.3. Chemical Analysis of Warmed-over Flavour

Methodology

The results from the sensory evaluation of reheated chill-stored cooked beef clearly demonstrated that quality reduction of this type of product was solely due to the development of WOA and WOF. Blade was shown to develop a higher level of WOF than loin and this may reflect differences in the lipid composition between the two muscles. In order to determine the source of WOA and WOF during chilled storage of cooked beef a number of different analyses, focussed on lipid chemistry, were performed on the same samples that had been evaluated by the taste panel.

(i) Lipid Content

Animal fats contain a variety of lipid components. Neutral lipids are fats (triglycerides) that are esters of glycerol containing three long chain fatty acids. Depot fat of adipose tissue consists mainly of neutral lipids but these lipids can also exist as fat globules within muscle cells, especially the more oxidative or red muscle fibre types.

Phospholipids are also esters of glycerol and, besides containing two long chain fatty acids, have a phosphate group attached to the glycerol backbone. Phospholipids are the principal structural components of the outer cell membrane and intracellular organelles such as mitochondria. Phospholipids generally contain high levels of polyunsaturated fatty acids which are required to maintain the cell membranes in a fluid state.

Total muscle lipids (consisting of neutral lipids and phospholipids) were extracted from the cooked beef slices using chloroform / methanol (2:1 by volume). The chloroform / methanol was evaporated from the extracts and the weight of the dry residue was expressed as percent total lipid (grams lipid per 100 grams cooked meat).

Neutral lipids and phospholipids were separated from the total lipid extracts by passage through silica gel columns. The neutral lipids do not "stick" to the column and are washed out using chloroform. The phospholipids are retained on the column during chloroform washing and were subsequently removed by washing the column with methanol. Analysis of phosphorous content of the methanol fraction allows for the quantification of the phospholipid fraction. The difference between the total lipid content and phospholipid content was defined as neutral lipid.

(ii) Thiobarbituric Acid Reactive Substances (TBARS)
Peroxides, formed from the oxidation of lipids, degrade to a variety of secondary reaction products including alcohols, aldehydes, ketones, and hydrocarbons. One product, indicative of lipid oxidation, is malondialdehyde (MDA), a three carbon dicarbonyl compound. Quantification of MDA by colorimetric assay of the pink-coloured complex that MDA forms upon reaction with thiobarbituric acid (TBA) is the most commonly used method for determining lipid rancidity in muscle foods.

Cooked meat samples, while still frozen, were pulverised to a fine powder in a blender. Accurately weighed samples of the meat powder were homogenised in trichloroacetic acid. The suspension was filtered and the MDA content of the filtrate was determined by measuring the absorbance of the pink reaction product at 532 nm in a spectrophotometer, following reaction with TBA for 15 minutes at 100°C. Standard solutions of pure MDA were used to calibrate the method.

(iii) Fatty Acid Analysis
Lipid oxidation results from the destruction of unsaturated fatty acids present in the various muscle lipid fractions. Therefore it is possible to measure the overall rate of the oxidation process by measuring the loss of each fatty acid. In theory, it should then be possible to relate the loss of a particular type of fatty acid to the increase in TBARS value.

The fatty acids in extracts containing the total lipids, neutral lipids and phospholipids from the sensory-evaluated cooked beef slices were released by disrupting the fats in methanol containing sulphuric acid at 60°C. This hydrolysis converts the fatty acids into volatile methyl esters which were subsequently separated by gas chromatography. The amount of each fatty acid was determined from the areas of each peak by comparison with known amounts of standard fatty acids.

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Results of chemical analyses

Lipid content

The total lipid, neutral lipid and phospholipid content of sensory samples did not change during the six days of chilled storage and none of the chilled samples differed from the frozen controls. However, the two muscles differed in the amount of lipids present and these differences are shown below in Figure 1.2.

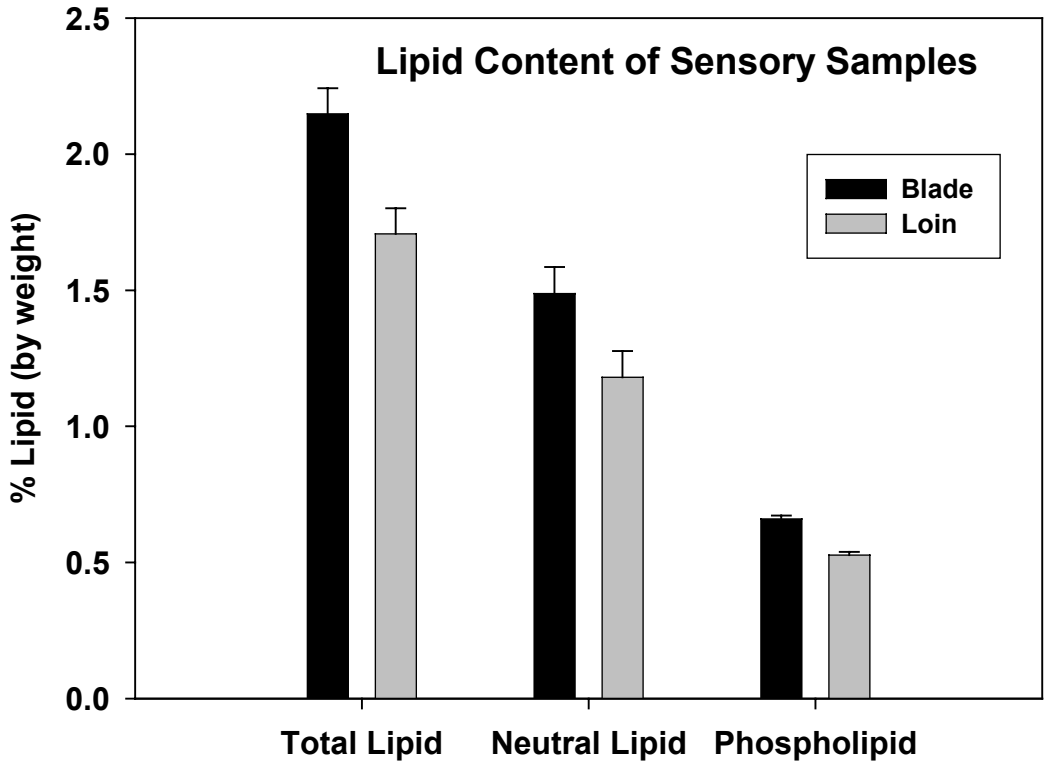

Figure 1.2. Total lipid, neutral lipid and phospholipid content of cooked blade and loin slices used in the sensory evaluation of warmed-over flavour development during chilled storage of sliced beef.

Blade contained 26% more total lipid than loin. Interestingly, in both the neutral lipids and phospholipid fractions the cooked blade samples also displayed 26% higher lipid content than the cooked loin samples. Since the sensory scores for WOF were higher for blade than loin in chill-stored cooked slices, the results of Fig. 1.2 suggest that the higher lipid content of blade could be a contributing factor to the corresponding higher WOF sensory scores of blade compared to loin.

Thiobarbituric acid reactive substances (TBARS)
The development of lipid rancidity, determined by TBARS value, in the sensory-evaluated cooked beef slices during chill-storage is shown in Figure 1.3.

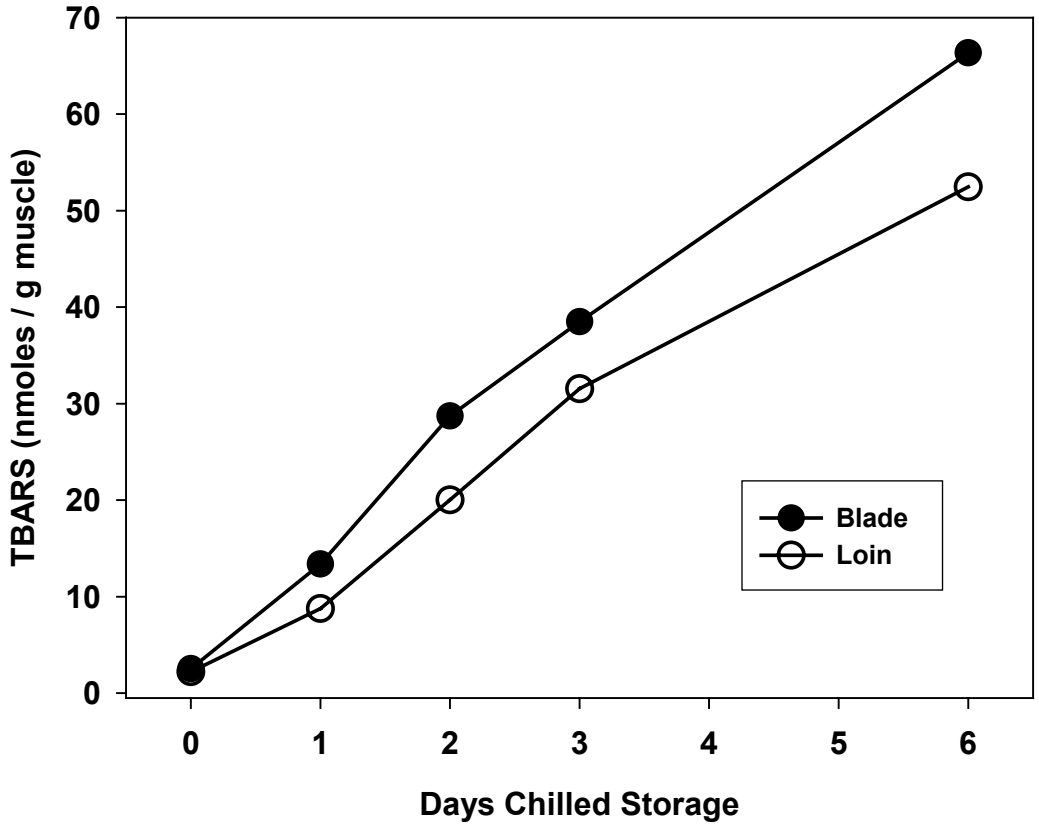

Figure 1.3. Development of lipid oxidation products in cooked sliced beef during chilled storage.

At each storage time from 1 day onwards the blade samples displayed higher levels of lipid oxidation (TBARS) than those observed for the loin samples. Overall, the blade samples had about 30% higher TBARS than the loin samples throughout the chilled storage period. This level of difference in the amount of lipid oxidation between the two muscles is in good agreement with the difference in lipid content (Fig. 1.2) found between these two muscles. The rate of TBARS development during chilled storage was similar for the two muscles.

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Fatty acid analysis

Figure 1.4 shows the effect of chilled storage on the levels of fatty acids in the cooked blade and loin sensory samples.

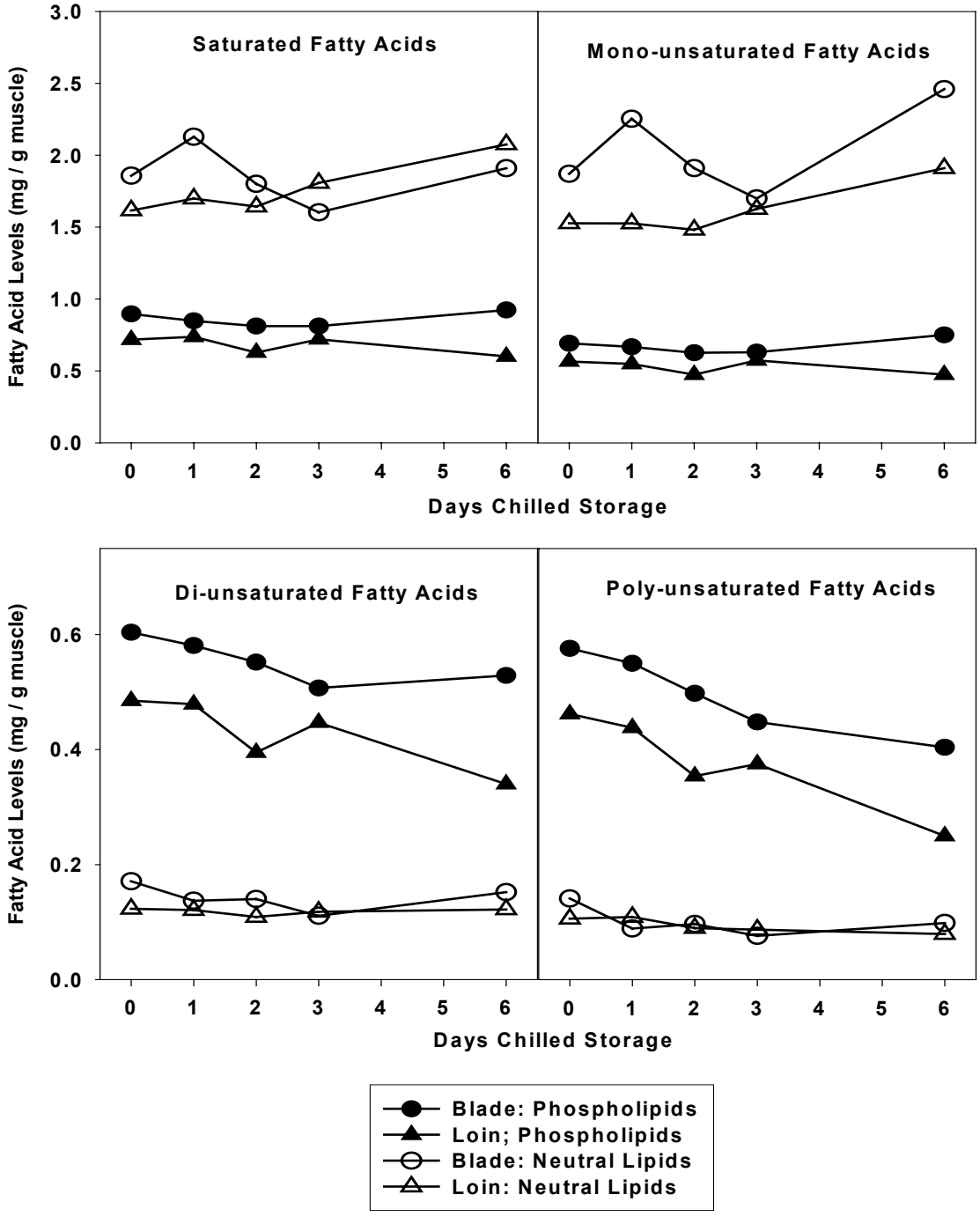

Figure 1.4. Fatty acid levels in cooked beef slices during chilled storage.

The level of fatty acids in the neutral lipid (open symbols in Fig. 1.4) and phospholipid (closed symbols Fig. 1.4) fractions from the sensory samples were examined. It is evident from Fig. 1.4 that the neutral lipid fraction, from both muscles (blade = open circles, loin = open triangles) contains higher levels of saturated and mono-unsaturated fatty acids than the phospholipid fraction (blade = closed circles, loin = closed triangles). On the other hand, the phospholipid fraction of both muscles contained much higher levels of di-unsaturated and polyunsaturated fatty acids than the neutral fraction.

The level of saturated fatty acids in the neutral fraction was not significantly different between the muscles and also did not change during chilled storage. This was expected to be the case since some degree of fatty acid unsaturation is required for oxidation to occur i.e. saturated fats couldn't oxidise under the mild conditions of chilled storage because they contain no double bonds for oxygen to attack. The level of saturated fatty acids in the phospholipid fraction also did not change during chilled storage but here, interestingly, the blade samples contained higher levels of saturated fatty acids (approximately 26%) than the loin samples.

The level of monounsaturated (one double bond per fatty acid) fatty acids did not change in either the neutral or phospholipid fractions during storage of the sensory samples. Again, no muscle differences were observed in monounsaturated fatty acid content in the neutral lipid fraction but in the phospholipid fraction blade samples showed higher levels of monounsaturated fatty acids compared to the loin samples.

The level of diunsaturated (two double bonds per fatty acid) fatty acids followed the same pattern seen for the saturated and monounsaturated fatty acids i.e., no significant change during storage in either fraction and higher levels in the phospholipid fraction of the blade sample compared to that of loin.

Levels of polyunsaturated (three or more double bonds per fatty acid) fatty acids were very low in the neutral lipid fraction and these levels did not change during storage nor did they differ between muscles. The levels of polyunsaturated fatty acids (PUFA) in the phospholipid fraction showed significant muscle differences and also significant decline with increasing chilled storage time.

Blade phospholipids consistently displayed 30% higher levels of PUFA compared to those of loin phospholipids at all storage times. The rate of loss of phospholipid PUFA in both muscles was similar and following 6 days chilled storage approximately 40% of the 0 day levels of PUFA were lost in the samples.

Six phospholipid PUFA were identified in sensory samples and these ranged from 18 to 22 carbon atoms in length and their level of unsaturation ranged from 3 to 6 double bonds. Details of these phospholipid PUFA are presented in Table 1.2.

Table 1.2. Phospholipid polyunsaturated fatty acids identified in blade and loin cooked beef slices.

Chain length:No of double bonds	Common name	Chemical name
18:3	α-Linolenic acid	9,12,15-octadecatrienoic acid
20:3	Dihomo-γ-linolenic acid	8,11,14-eicosatrienoic acid
20:4	Arachidonic acid	5,8,11,14- eicosatetraenoic acid
20:5	Timnodonic acid	5,8,11,14,17- eicosapentaenoic acid
22:5	Clupanodonic acid	7,10,13,16,19-docosapentaenoic acid
22:6	Cervonic acid	4,7,10,13,16,19-docosahexaenoic acid

Of the six phospholipid PUFA, 18:3 and 20:3 did not decline during chilled storage of the cooked beef slices. Rates of decline for the other four PUFA during chilled storage is shown below in Figure 1.5.

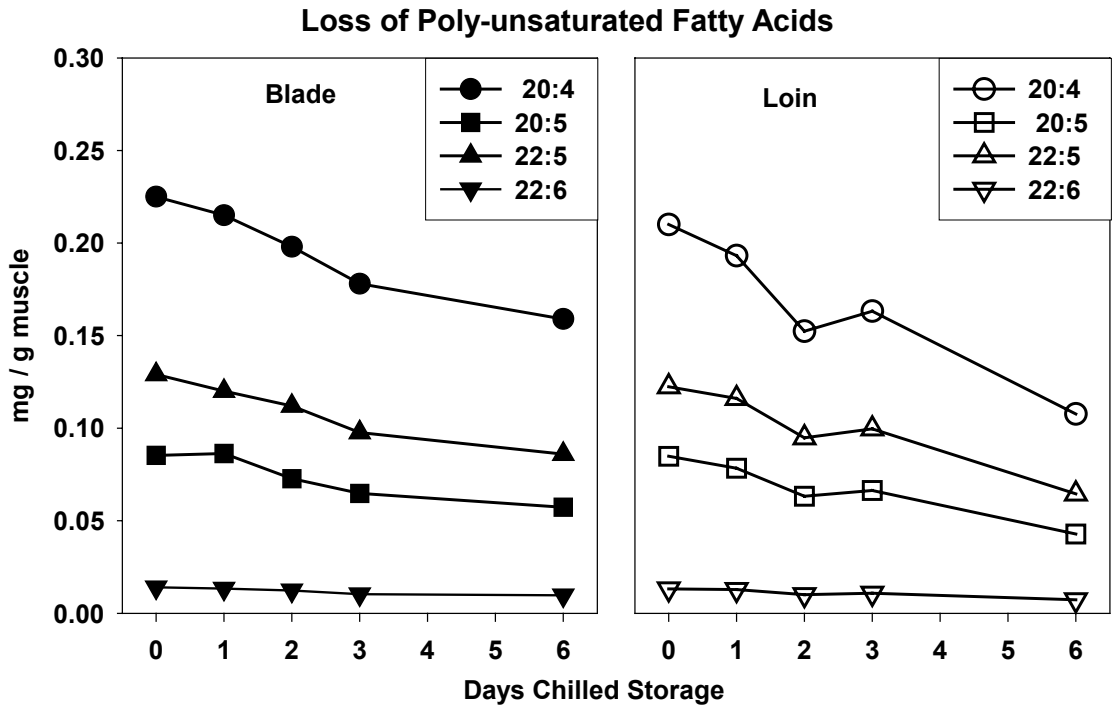

Figure 1.5. Loss of phospholipid polyunsaturated fatty acids during chilled storage of blade and loin cooked beef slices.

Of the four phospholipid PUFA that declined during chilled storage of cooked beef, arachidonic acid (20:4) was the most abundant accounting for 50% of their total sum in both muscles. 22:5 was next with 28% of total, followed by 20:5 (19% of total) and 22:6 was the least abundant (3% of total). The blade samples had 30% more of each of the four phospholipid PUFA than that found in the corresponding loin samples.

1.4. Relationships between sensory and chemical evaluation of warmed-over flavour.

Relationship between loss of PUFA and development of lipid oxidation products (TBARS).

In order to establish that the loss of four phospholipid PUFA (Fig.1.5) was related to the observed increase in lipid oxidation products (Fig. 1.3), during chilled storage of cooked beef, the loss of each of the four phospholipid PUFA was plotted against the corresponding amount of lipid oxidation products (TBARS value) in that sample. For this exercise the loss of PUFA was defined as the amount present in the frozen control sample (0 day chilled storage) minus the amount at each time point of chilled storage. These calculations were performed for each of the four individual PUFA as well as for the summed loss of the four PUFA. The results for the latter are shown below in Figure 1.6.

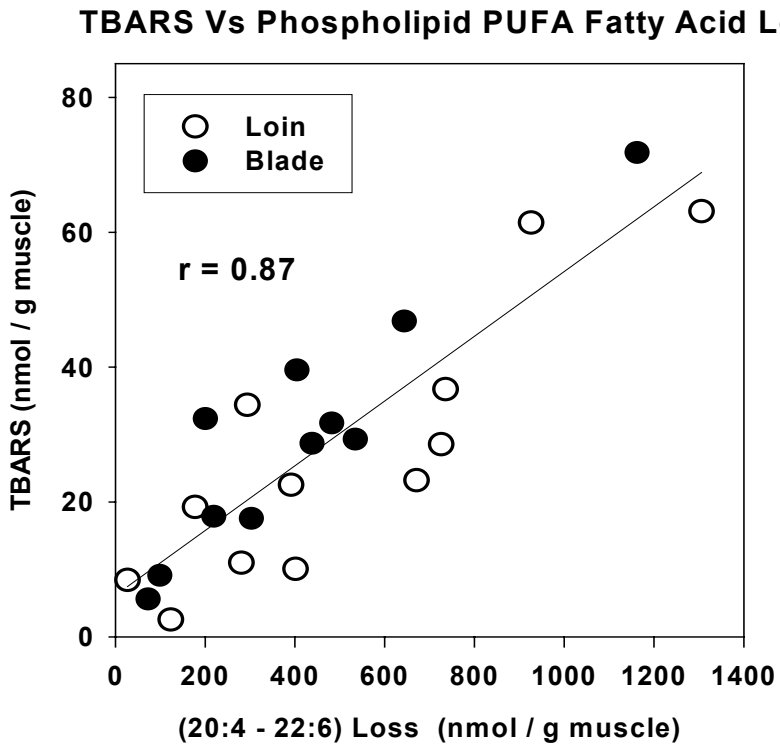

Figure 1.6. Relationship between polyunsaturated fatty acid loss and development of lipid oxidation products in cooked beef during chilled storage.

Regression analysis of the data presented in Fig. 1.6 resulted in a correlation coefficient of r = 0.87, (r ranges from 0 to 1, with 0 indicating no relationship and 1 that of a perfect fit), indicating a strong positive relationship between the loss of the four PUFA that declined during storage and the amount of lipid oxidation products formed at that corresponding timepoint.

Each of the four individual PUFA gave very similar results to that of the sum of the four as shown in Fig. 1.6. The correlation coefficients for 20:4, 20:5, 22:5 and 22:6 were 0.83, 0.83, 0.89 and 0.89, respectively.

Relationship between warmed-over flavour and aroma sensory scores and the amount of chemically determined lipid rancidity (TBARS value)
Having established that the loss of four phospholipid PUFA was related to the onset of lipid rancidity (TBARS value) the next step was to determine if the sensory scores for warmed-over flavour and aroma related to the chemical values for lipid rancidity. The plots of this data are shown below in Figure 1.7.

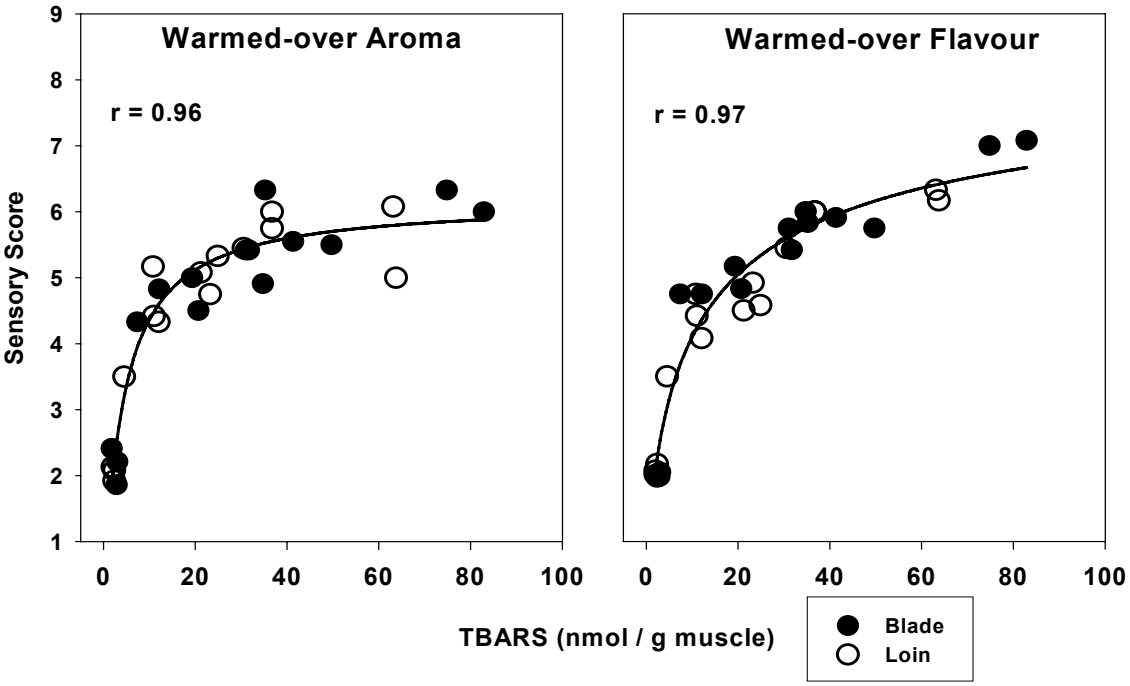

Figure 1.7. Relationship between sensory scores for warmed-over aroma and flavour and chemically determined lipid rancidity (TBARS).

The data points in Fig. 1.7 indicated a curvilinear relationship between chemically determined rancidity (TBARS values) and sensory scores for warmed-over aroma and flavour. It appeared that as the TBARS value increased the corresponding increase in sensory score became less.

Furthermore, it appeared that above a certain TBARS value no more sensory response could be evoked i.e., saturation of the olfactory and flavour senses had occurred. This effect was particularly evident for the warmed-over aroma scores.

Such behaviour is common in biological systems and can be mathematically described by the Hill Equation. This equation describes co-operative binding of several molecules to receptor sites (usually on a protein). Binding of one molecule will elicit a small response and as more molecules bind a dramatic increase in response is observed but as more molecules bind the sites become saturated and maximal response is reached. It is known that olfactory and flavour receptor proteins behave in this manner.

The curves fitted in Fig. 1.7 were calculated using the Hill Equation on the data points and as can be seen the fit was excellent. Warmed-over aroma and flavour gave correlation coefficients of r = 0.96 and 0.97, respectively, with TBARS values. This result indicated that the products formed from the oxidation of four phospholipid PUFA can account for virtually all of the off-flavours and aromas detected by sensory evaluation during reheating of chilled cooked beef.

Analysis of the Hill plots in Fig. 1.7 allowed several important parameters to be determined. One of these was the degree of chemically determined lipid rancidity at which half-maximal sensory response was reached. This TBARS value turned out to be 4.08. From the rate of TBARS development (Fig. 1.3) a TBARS value of 4.08 in cooked beef would be reached following 5 hours of chilled storage. This showed how rapidly undesirable flavours and aromas can develop in cooked beef during chilled storage. Information of this type would be very important to manufacturers of cooked ready-to-eat convenience beef products. The TBARS value of a cooked meat product is simple and rapid to perform and, as shown from the results of this study, correlated extremely well with sensory data.

1.5. Reference material

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2. Characterisation of muscle phospholipid composition.

2.1. Background

It is known that polyunsaturated fatty acids such as arachidonic acid are found predominantly in membranes surrounding intracellular organelles such as mitochondria and endoplasmic reticulum. In muscle cells the endoplasmic reticulum has become highly specialised in uptake and release of calcium ions during contraction and relaxation activity and is called the sarcoplasmic reticulum.

In biological membranes the polyunsaturated fatty acids are chemically bound to glycerol and phosphate and in this form are known as phospholipids. Depending on the nature of the phosphate group the phospholipids are assigned to four major classes. Within each phospholipid class the attachment of fatty acids to the glycerol portion of the molecule can occur in different positions giving rise to different molecular species within the same class, adding a further layer of complexity to the structure of these compounds.

Not much research has focussed on the composition and structure of muscle phospholipids to date and even less on bovine muscle phospholipids. Muscle tissue is rich in a special class of phospholipids known as plasmalogens. In most phospholipids both long chain fatty acids are attached to the glycerol moiety by ester linkages but in plasmalogens one of the fatty acids is linked to glycerol by a vinyl ether bond. This unique chemical structure of plasmalogens is postulated to protect the long chain unsaturated fatty acids from oxidation.

The objective of this part of the project was to define the phospholipid composition of sarcoplasmic reticulum and mitochondrial membranes from fore- and hindquarter muscles in order to determine their relative contributions to WOF.

2.2. Experimental design and procedure

Since high value pre-cooked "warm and eat" convenience beef meals would be generally prepared from lower value beef forequarter cuts it was decided to compare and contrast two forequarter cuts with primal hindquarter tenderloin (LD). The forequarter cuts were selected to also contrast forequarter muscle fibre type differences. For these reasons the *infraspinatus* muscle (oyster blade) and the *biceps brachii* muscle (part of shin beef or conical muscle when sold as a separate cut) were the selected experimental forequarter muscles. The *infraspinatus* (IN) muscle consists predominantly of slow oxidative muscle fibres (80%-90%) while the *biceps brachii* (BB) is a fast oxidative muscle.

Six Hereford cattle of 429 ± 9 kg live weight were used in this experimental work. The cattle were sourced from the Darling Downs and were grain fed prior to transport to Cannon Hill. Animals were killed by captive bolt and dressed at the abattoir facility of Food Science Australia's Brisbane Laboratory (Cannon Hill). Muscle core samples (approximately 20 grams) were removed from the IN, BB and LD muscles at approximately 45 minutes post-mortem.

Mitochondrial (Mito) and sarcoplasmic reticulum (SR) membranes were prepared from the muscle samples and the isolated membrane fractions were snap frozen in liquid nitrogen and then stored at -70°C until the membrane lipids were extracted. Membrane phospholipids (PL) were extracted from Mito and SR using chloroform / methanol as solvent. Membrane PL classes were prepared from the PL extracts using a high performance liquid chromatography system and each PL class fatty acid composition was determined by a gas chromatographic procedure.

Significance of differences was determined using two way analysis of variance for muscle (IN, BB and LD) by membrane (Mito and SR).

2.3. Mitochondrial and sarcoplasmic reticulum content of forequarter muscles compared to tenderloin

The total membrane fraction of muscle homogenates was collected by centrifugation at 100,000 *g*. This membrane fraction was then run on sucrose density gradients and the Mito and SR fractions were collected at the 38%-50% and 24%-38% sucrose interfaces, respectively (Figure 2.1).

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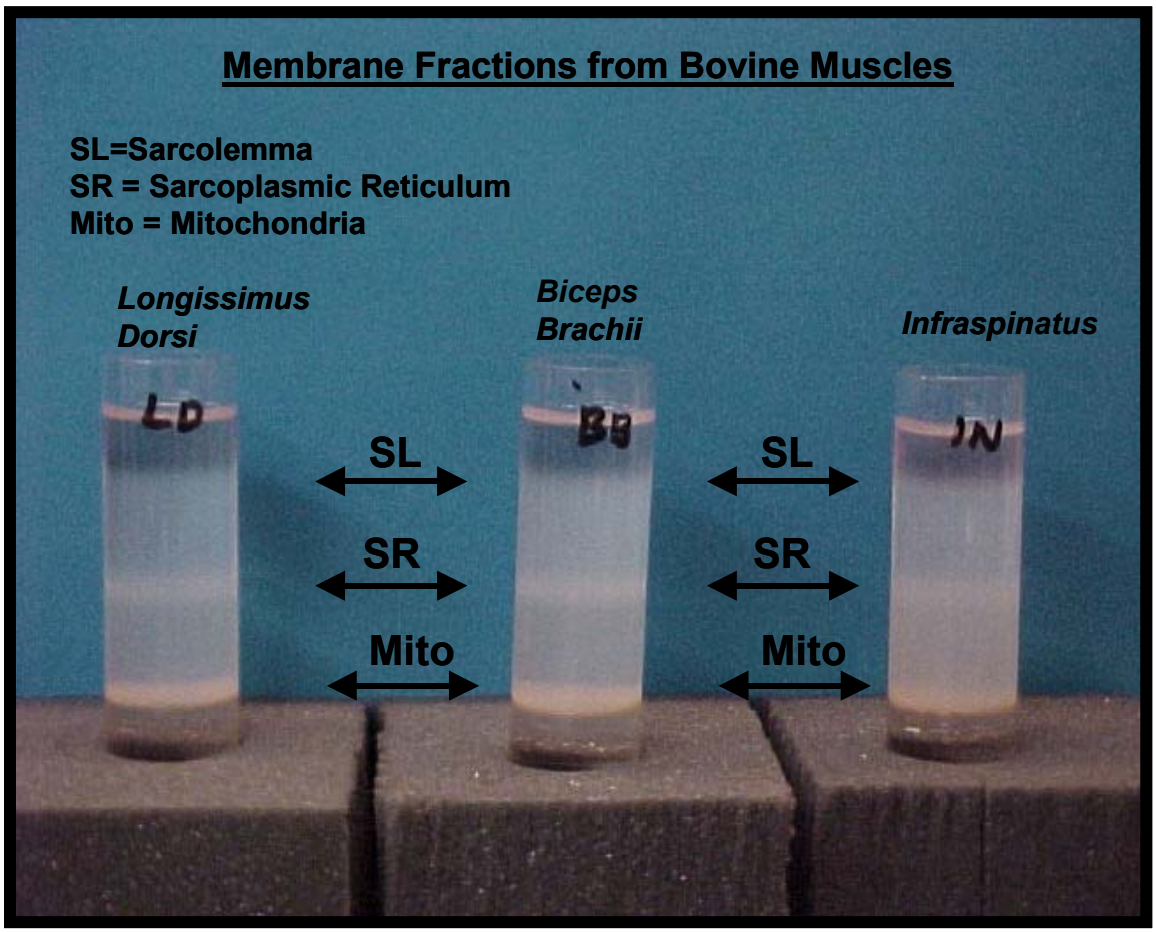

Figure 2.1. Separation of mitochondrial (Mito) and sarcoplasmic reticulum (SR) membrane systems from tenderloin (LD) and forequarter muscles *biceps brachii* (BB) and *infraspinatus* (IN).

The membrane yields (mg / gram of muscle) of the different muscles are presented in Table 2.1.

Table 2.1. Yield of muscle membrane (mg / gram of muscle) from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results are least square means for muscle X membrane. The pooled estimate for the standard error of the mean was ± 0.076.

Muscle	Membrane	
	Mitochondria	Sarcoplasmic reticulum
IN	1.124	0.600
BB	1.136	0.462
LD	0.923	0.477

Statistical analysis indicated that the yields of both membrane systems did not differ significantly between muscles. However, the difference in Mito and SR yields within each muscle was highly significant. In all of the muscles the yield of M was approximately twice that of SR.

2.4. Total phospholipid content of mitochondrial and sarcoplasmic reticulum membranes of bovine forequarter and tenderloin muscles.

Total phospholipids were extracted from muscle membrane preparations using a chloroform / methanol extraction protocol and the phospholipid content of the extracts was then determined by phosphorus analysis. Results in nanomoles of membrane phospholipid per milligram of membrane protein are given in Table 2.2.

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Table 2.2. Total phospholipid content of mitochondrial (Mito) and sarcoplasmic reticulum (SR) membrane systems from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results (nanomoles of phospholipid per milligram of membrane protein) are least square means for muscle X membrane ± standard error of the mean.

Muscle	Membrane	
	Mitochondria	Sarcoplasmic reticulum
IN	884.10 ± 41.70	601.28 ± 41.70
BB	849.50 ± 41.70	647.03 ± 46.62
LD	831.77 ± 53.83	654.83 ± 53.83

Statistical analysis indicated that the phospholipid content of both membrane systems did not differ significantly between muscles. However, the difference between Mito and SR phospholipid within each muscle was highly significant. In all of the muscles the phospholipid content of Mito was approximately 35% higher than that of SR.

2.5. Phospholipid class composition of mitochondrial and sarcoplasmic reticulum membranes from forequarter and tenderloin muscles.

The structure of membrane phospholipids is based on a glycerol backbone in which the three hydroxyl groups are esterified to two fatty acids at positions 1 and 2, and a phosphate group at position 3. Different phospholipid classes are defined by the chemical nature of the head group attached to the phosphate group. In general saturated fatty acids occur in the 1 position while unsaturated fatty acids always occur in the 2 position. These points are illustrated in Figure 2.2.

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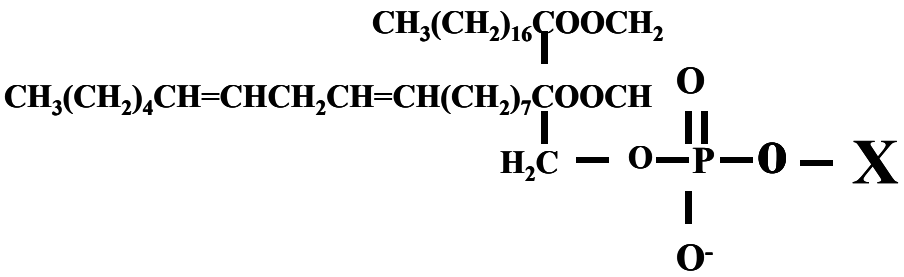
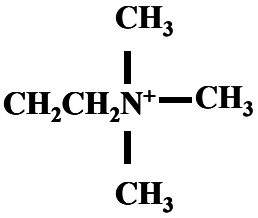


Phosphatidylcholine (PC) $X =$ 

Phosphatidylethanolamine (PE) $X = CH_2CH_2N^+H_3$

Phosphatidylserine (PS) $X = CH_2CH(N^+H_3)COO^-$

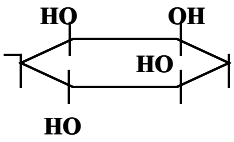
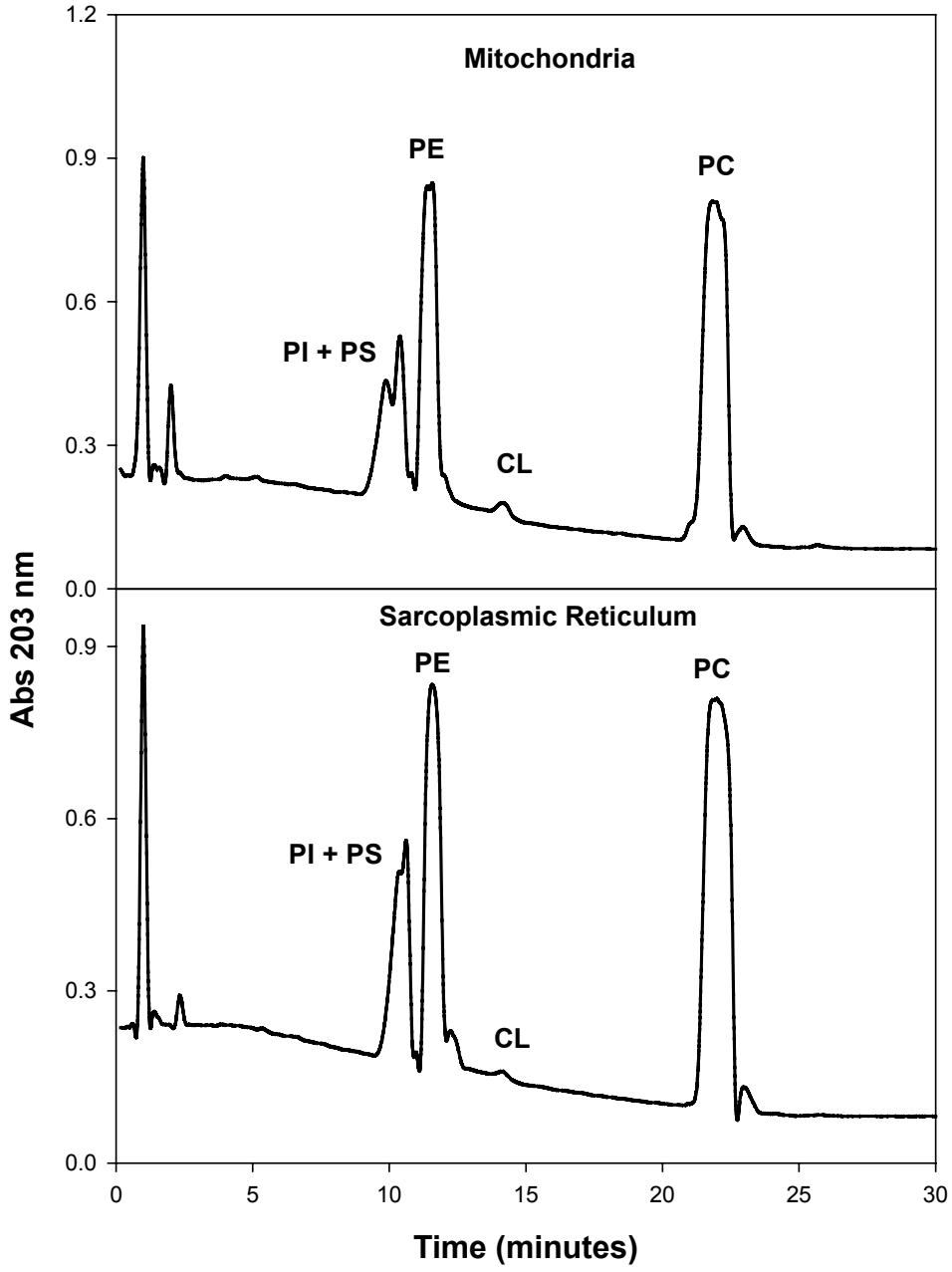
Phosphatidylinositol (PI) $X =$ 

Figure 2.2. Phospholipid structures. Top structure illustrated has a C18 saturated fatty acid in the 1 position of the glycerol backbone and an 18:2 unsaturated fatty acid in the 2 position. X represents the head group attached to the phosphate group and below the general phospholipid structure are the structures of the 4 main head groups that define the phospholipid classes.



HPLC separation of phospholipid classes.
Figure 2.3. HPLC separation of phospholipid classes.

The phospholipid classes were separated in the total lipid extract of each membrane system by a high performance liquid chromatographic procedure. A chromatogram illustrating the separation of the major phospholipid classes in mitochondria and sarcoplasmic reticulum is presented in Figure 2.3.

In general, PC is the most abundant phospholipid class found in biological membranes and accounts for approximately 60%-75% of the total phospholipids. PE is the next most abundant class, accounting for approximately 15%-20%. PI and PS are the least abundant PL classes with their combined sum usually not exceeding 10% of the total PL fraction. Cardiolipin (CL) is a diphosphatidylglycerol localized in the inner mitochondrial membrane. It accounts for less than 10% of the total PL fraction in mitochondria and is unlikely to play a role in WOF development because 90% of its fatty acid composition consists of diunsaturates (18:2).

While PC is the most abundant PL class, PE generally contains the highest levels of PUFAs of the PL classes and is therefore a likely source of oxidisable fatty acids leading to the development of WOF.

The mole percent of PC, PE, PI/PS and CL for the 2 membrane systems in the three muscles is given below in Table 2.3.

Table 2.3. Phospholipid class distribution (mole % of total) of mitochondrial (Mito) and sarcoplasmic reticulum (SR) membrane systems from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results are presented as least square means for muscle X membrane ± standard error of the mean.

	Phospholipid Class							
	PC		PE		PI/PS		CL	
	membrane		*membrane*		*membrane*		*membrane*	
Muscle	***Mito***	***SR***	***Mito***	***SR***	***Mito***	***SR***	***Mito***	***SR***
IN	50.38	64.20	24.12	20.30	10.92	9.62	2.42	0.49
	± 3.69	± 3.9	± 2.39	± 2.39	± 0.95	±0.95	± 0.57	±0.57
BB	58.16	61.60	32.38	20.10	7.63	4.43	4.29	2.53
	± 3.69	± 4.13	± 2.39	± 2.68	± 1.06	± 1.23	±0.57	± 0.63
LD	50.93	56.73	23.30	15.30	9.57	4.60	4.08	1.70
	± 4.77	± 4.77	± 3.09	± 3.09	± 1.23	± 1.23	± 0.73	± 0.73

The four PL classes of PC, PE, PI/PS and CL accounted for approximately 90% of the total PL recovered from the column.

The PC content did not differ significantly between the muscles but the SR had higher PC content than Mito. BB muscle tended to have the highest PE

and this difference was close to being significant. SR had significantly less PE than M. The IN muscle had significantly higher levels of PI/PS than BB and LD and PI/PS was also significantly higher in Mito compared to SR. CL was much higher in M than SR, as was expected since CL is a mitochondrial phospholipid. Interestingly, the levels of CL were higher in M of BB and LD compared to IN.

2.6. Fatty acid composition of muscle membrane phospholipid classes.

The fatty acid profiles of the phospholipid classes for Mito and SR membranes from IN, BB, and LD muscles were determined by gas chromatographic analysis. The results of these analyses for PC, PE, and PI/PS are presented in Tables 2.4, 2.5 and 2.6, respectively.

Table 2.4. Fatty acid composition for PC phospholipid class (% of total) of mitochondrial (Mito) and sarcoplasmic reticulum (SR) membrane systems from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results are presented as least square means for muscle X membrane ± standard error of the mean.

	Muscle					
	IN		BB		LD	
Fatty acid	*membrane*		*membrane*		*membrane*	
	SR	***Mito***	***SR***	***Mito***	***SR***	***Mito***
saturated	28.4	27.3	28.6	27.8	29.8	29.3
	± 0.7	± 0.7	± 0.8	± 0.7	± 0.9	± 0.9
monounsaturated	39.6	40.7	37.0	41.5	41.2	43.2
	± 1.2	± 1.2	± 1.3	± 1.2	± 1.5	± 1.5
diunsaturated	16.8	17.1	16.7	16.1	14.9	14.2
	±1.9	± 1.9	± 2.1	± 1.9	± 2.4	± 2.4
polyunsaturated	15.2	14.9	16.5	15.6	14.1	13.4
	± 1.4	± 1.4	± 1.6	± 1.4	± 1.8	± 1.8
arachidonate (20:4)	4.9	4.7	5.2	5.1	4.1	3.9
	± 0.3	± 0.3	± 0.4	± 0.3	± 0.4	± 3.9

The total saturated fatty acids, di- and PUFAs in PC (Table 2.4) showed no significant differences for muscles or membranes. However, Mito did show significantly higher levels of monounsaturated fatty acids than SR, but the difference was not large. The BB muscle showed significantly higher levels of

arachidonate in both SR and Mito than the other two muscles but again the difference was not large.

Table 2.5. Fatty acid composition for PE phospholipid (% of total) of mitochondrial (Mito) and sarcoplasmic reticulum (SR) membrane systems from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results are presented as least square means for muscle X membrane ± standard error of the mean.

	Muscle					
	IN		BB		LD	
Fatty acid	*membrane*		*membrane*		*membrane*	
	SR	***Mito***	***SR***	***Mito***	***SR***	***Mito***
saturated	15.6	26.9	18.2	26.6	12.1	24.3
	± 1.8	± 1.8	± 2.0	± 1.8	± 2.3	± 2.3
monounsaturated	14.3	12.2	14.1	13.7	16.1	14.7
	± 0.7	± 0.7	± 0.8	± 0.7	± 0.9	± 0.9
diunsaturated	15.5	12.1	13.6	13.7	16.2	12.7
	± 1.7	± 1.7	± 1.9	± 1.7	± 2.2	± 2.2
polyunsaturated	53.1	49.1	48.8	45.9	55.6	48.4
	± 2.2	± 2.2	± 2.4	± 2.2	± 2.8	± 2.8
arachidonate (20:4)	24.0	27.4	22.6	24.1	21.0	23.3
	± 1.3	± 1.3	± 1.5	± 1.3	± 1.7	± 1.7

Saturated, monounsaturates, and PUFAs of PE (Table 2.5) all showed significant membrane differences. Mito had higher levels of saturated fatty acids than SR, while the reverse was the case with the mono- and polyunsaturates. No significant differences were observed in diunsaturated fatty acids between muscles or membranes. The only significant muscle difference for the PE fatty acids occurred in the monounsaturates, where the LD has a higher level than the other two muscles. Interestingly, while the total PUFAs showed significant membrane differences, the content of the major PUFA of PE, arachidonate (20:4), was not significantly different between muscles or membranes.

When the fatty acid profiles of PC (Table2. 4) are compared to those of PE (Table 2.5) some large class differences are readily apparent. There are much higher overall levels of saturates and monounsaturates in PC compared to PE. The substantially higher levels (approximately three fold) of PUFAs in PE are also quite striking. This difference is even greater in arachidonate, the

major PUFA of both classes, where a five fold difference is evident between PE and PC. Interestingly, the levels of diunsaturates are quite similar between the two classes.

Table 2.6. Fatty acid composition for PI/PS phospholipid (% of total) of mitochondrial (Mito) and sarcoplasmic reticulum (SR) membrane systems from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results are presented as least square means for muscle X membrane ± standard error of the mean.

	Muscle					
	IN		BB		LD	
Fatty acid	*membrane*		*membrane*		*membrane*	
	SR	***Mito***	***SR***	***Mito***	***SR***	***Mito***
saturated	38.4	25.9	30.6	14.6	45.2	25.2
	± 3.9	± 3.9	± 5.1	± 4.4	± 5.1	± 5.1
monounsaturated	17.8	20.8	18.9	23.7	16.6	21.5
	± 2.0	± 2.0	± 2.6	± 2.3	± 2.6	± 2.6
diunsaturated	16.3	31.8	19.8	42.2	8.8	31.7
	± 3.7	± 3.7	± 4.8	± 4.2	± 4.8	± 4.8
polyunsaturated	27.5	21.5	30.8	19.5	29.4	21.7
	± 1.4	± 1.4	± 1.9	± 1.6	± 1.9	± 1.9
arachidonate (20:4)	9.8	6.8	10.0	4.8	12.2	7.0
	± 1.5	± 1.5	± 1.9	± 1.6	± 1.9	± 1.9

The fatty acid profile of PI/PS (Table 2.6) revealed only one significant muscle difference and that was the lower level of saturates in the BB muscle compared to IN and LD. In all the fatty acids of PI/PS highly significant membrane differences were apparent. SR had higher levels of saturates than Mito but the latter had higher levels of monounsaturates than SR. The largest membrane difference in the PI/PS class was in the level of diunsaturates where M displayed approximately twice the level of SR. The total PUFAs were marginally higher in SR than Mito but the difference in arachidonate between membranes was much larger than this, with 70% higher levels in SR compared to Mito.

Compared to PC and PE classes, the level of PI/PS PUFAs falls intermediate between the low levels of PC and the very high levels seen in PE. Diunsaturates in all classes were of similar overall levels. PI/PS tended to

have comparable levels of saturates to PC while monounsaturate levels were closer to those of PE.

Overall, very few muscle differences were found in fatty acid composition of the PL classes. This was somewhat surprising in view of the differences in anatomical location and reported fibre type differences of the muscles examined in this study. On the other hand, large differences were observed between the membranes of SR and Mito, especially in the PE class, with SR generally tending to have higher levels of PUFAs than Mito.

2.7. Estimated potential warmed-over flavour contribution of muscle membranes

In Section 1.1, the development of WOF was shown to arise predominantly from membrane PUFAs. This correlation was particularly strong with arachidonate (20:4), the major PUFA found in mammalian membranes. Therefore, it could be postulated that the higher the levels of arachidonate present in a membrane system, the higher the likelihood of WOF development.

In order to assess the WOF potential of the three muscles and the membrane systems within them, the amount of arachidonate per gram of muscle for each PL class was calculated. The first step of this calculation was to determine the total PL content for each membrane. This was done by multiplying the membrane yields (Table 2.1) by the total PL content of that membrane (Table 2). This resulted in a value of total membrane PL per gram of muscle. The content of arachidonate for each PL class was then determined by multiplying the total membrane PL by the fraction of the PL class in that membrane (Table 2.3) and also by that class fraction of arachidonate (Tables 2.4-2.6). For example, the arachidonate content of PC in SR from IN muscle would be 0.6 mg SR/gram muscle (yield of SR from IN, Table 2.1) X 601.3 nanomoles PL / mg SR (PL content of SR from IN, from Table 2.2) X 0.642 (PC fraction of SR in IN, from Table 2.3) X 0.049 (arachidonate fraction in PC from IN SR, Table 2.4) = 11.4 nanomoles arachidonate per gram of muscle.

The arachidonate content, on a weight of muscle basis, calculated as explained above for the PL classes of membranes within muscles are presented in Table 2.7.

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Table 2.7. Arachidonate (nanomoles / gram muscle) for phospholipid classes of sarcoplasmic reticulum and mitochondrial membrane systems from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Results are presented as least square means ± standard error of the mean. Total is the sum of arachidonate across each row.

	Membrane						
	Sarcoplasmic reticulum			Mitochondria			
Muscle	**PC**	**PE**	**PI/PS**	**PC**	**PE**	**PI/PS**	**Total**
IN	11.0	18.6	3.5	23.8	64.3	7.3	128.5
	± 1.7	± 3.7	± 0.8	± 2.6	± 6.6	± 1.0	± 7.5
BB	10.9	14.9	1.7	28.2	74.9	4.5	121.1
	± 1.7	± 2.1	± 0.8	± 3.5	± 12.2	± 2.4	± 14.9
LD	7.0	10.1	1.9	15.1	42.9	5.1	82.0
	± 1.5	± 3.9	± 0.9	± 1.5	± 10.7	± 0.4	± 18.4

Examination of Table 2.7 clearly shows that for each membrane system, PE has the highest arachidonate content and therefore oxidation of this PL class would be expected to be the major source of WOF compounds. Mitochondria are expected to be a much greater potential contributor to WOF than SR because it contains approximately 80% of the total arachidonate (SR + Mito). Indeed, in each muscle mitochondria PE accounts for approximately 50% of the total arachidonate.

Within the major PL classes of PC and PE of both membrane systems the LD muscle has less arachidonate than the forequarter IN and BB muscles. When comparing the total muscle arachidonate content, statistical analysis indicates that there is a 92% probability that the arachidonate levels of the LD are significantly lower than the two forequarter muscles. Therefore it seems likely that forequarter muscles have a greater WOF potential than the primal hindquarter cuts.

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3. Oxidative susceptibility of phospholipids within the major muscle membrane systems

3.1. Background

The results of the previous two Sections had shown that arachidonic acid (AA, 20:4) was the major polyunsaturated fatty acid of muscle membrane systems. This fatty has been reported to be very susceptible to oxidation. Oxidative degradation of AA in red meat and red meat products causes flavour deterioration through the development of stale and rancid off-flavours. The volatile odour-active compounds from AA oxidation have recently been characterised and are described as green, metallic, fatty and fishy.

We determined, in section 2, that the majority of AA in muscle membrane systems was found in phosphatidylethanolamine (PE) phospholipids. Furthermore, we also found that mitochondrial membranes (Mito) contained more PE phospholipids than the sarcoplasmic reticulum (SR) membrane. This was due to the additive effect of the higher membrane content plus higher lipid content of Mito in muscle compared to that of SR. On a muscle weight basis, these differences meant that Mito has up to five times more AA phospholipids than SR.

Currently, most models of muscle phospholipid oxidation have utilised muscle microsomes as a phospholipid source. Since muscle microsomes are predominantly SR our results, showing that Mito is a larger reservoir of AA than SR, would suggest that muscle microsomes are not the best choice as a model for muscle phospholipid oxidation studies. Use of SR for phospholipid oxidation studies is further complicated by the fact that SR is rich in plasmalogens, a unique subclass of phospholipids characterised by the presence of a vinyl ether substituent at the 1-position of the glycerol backbone. Several recent studies have shown that the fatty acids in the 2-position of the glycerol backbone in plasmalogens are more resistant to oxidation than their counterparts displaying the ester linkage at the 1-position. The vinyl ether moiety is also postulated to be a powerful free radical trap thereby rendering plasmalogen phospholipids more resistant to fatty acid oxidation than phospholipids that are exclusively ester-linked to the glycerol backbone.

The objective of Section was to investigate the oxidative susceptibility of Mito and SR phospholipids and thereby gain insight as to how the respective phospholipid molecular structures impact on their oxidation reactions.

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3.2. Experimental design and procedures

Overall oxidative susceptibility and AA oxidation of SR and Mito phospholipids was investigated using liposomes prepared from lipid extracts of Mito and SR isolated from *infraspinatus* (IN), *biceps brachii* (BB), and tenderloin (LD) bovine muscle. Liposomes are small membrane vesicles formed when dried phospholipid extracts are sonicated in physiological saline solutions. The phospholipids in liposomes orient themselves in the same manner as in "natural" membranes and are therefore excellent models for oxidation studies.

In order to initiate oxidation in liposomes a free radical generating system is added. However, there exists in the literature a plethora of methods for free radical generation suitable for the study of lipid oxidation. We decided on an iron / hydrogen peroxide system for initiating lipid oxidation, a method extensively investigated by Dr Steven Aust and his group at Michigan State University. Based on their research, we believe that this system simulates most closely the reactions thought to occur in post-mortem muscle and was therefore used in our experiments.

The extent of overall oxidation with time in liposomes was followed by reaction of phospholipid fatty acid degradation products with thiobarbituric acid (TBA). This reaction produces a pink colour that can be measured and quantitated as thiobarbituric acid reactive substances (TBARS). Samples from the same reaction were also subjected to fatty acid analysis by gas chromatography which enabled loss of specific fatty acids, such as AA, to be determined.

The molecular composition of individual phospholipid species comprising the PE class of Mito and SR was determined by high performance liquid chromatography (HPLC). The method we use separates the individual PE molecular species according to effective chain lengths of the fatty acids in the 1- and 2-positions and can also separate plasmalogens from conventional phospholipids.

3.3. Oxidative susceptibility of mitochondrial and sarcoplasmic reticulum membranes.

Freshly prepared liposomes from Mito and SR lipid extracts were incubated at 37°C in saline solution. At zero time, lipid oxidation was initiated by the addition of a defined amount of iron / hydrogen peroxide solution and the reaction was followed over a two hour time course. At selected time points the reaction was terminated and the amount of lipid degradation was determined as thiobarbituric acid reactive substances (TBARS). At 60 minutes samples were also assayed by gas chromatography for specific fatty acid degradation.

TBARS reached a plateau following 2 hours reaction and the pooled results (from IN and BB muscles) for SR and Mito are presented in Figure 3.1.

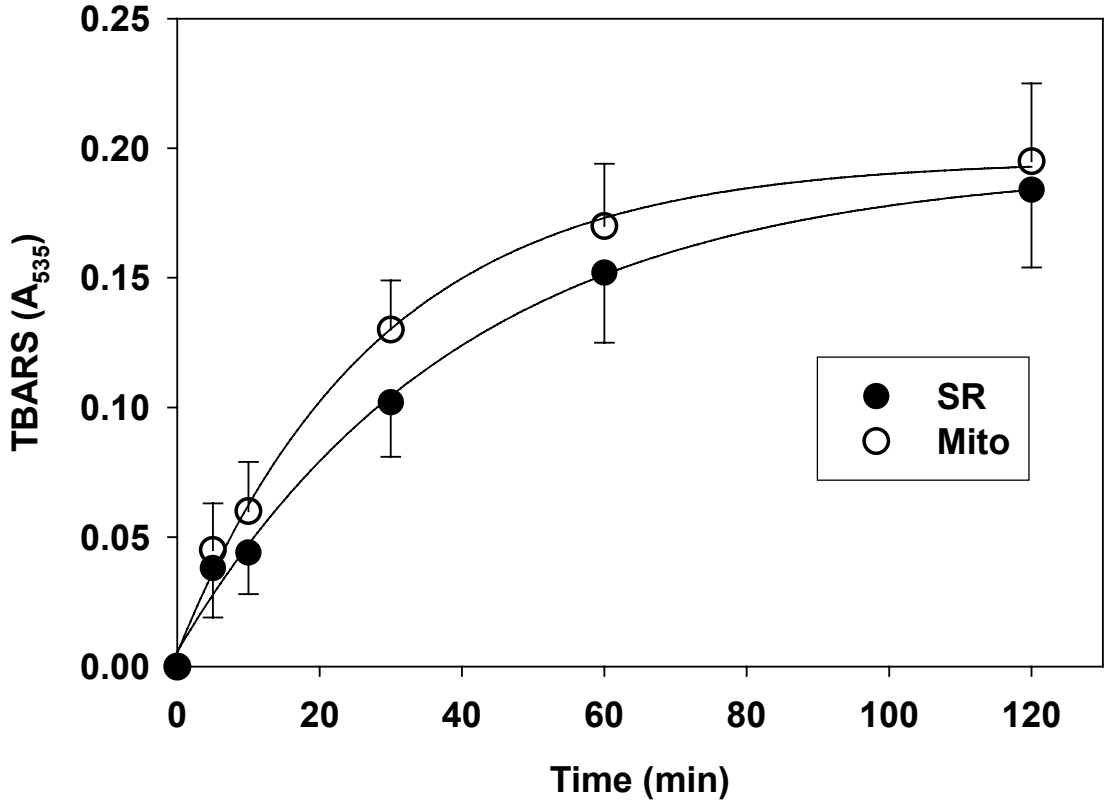

Figure 3.1. Oxidative degradation rates of SR and Mito phospholipids determined as TBARS. Each time point represents the mean ± standard deviation of 9 (SR) and 10 (Mito) samples. The fitted curve is that of exponential increase to a maximum value.

The TBARS data gave an excellent fit to an equation of exponential increase reaching a maximum value. From the fitted curves the rate constants for the reaction were calculated. Unfortunately, one of the LD SR extracts was lost and since there were only three LD preparations used in this study the LD results were excluded from the statistical analysis of the rate constants.

For IN and BB muscles the rate of lipid oxidation was significantly faster in Mito compared to SR. Since no muscle differences were observed (between IN and BB) the pooled muscle (IN + BB) results are shown in Figure 3.1.

The average oxidation rate constants for each muscle X membrane are presented in Table 3.1.

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Table 3.1. Rate constants (hr^{-1}) for oxidation of mitochondrial and sarcoplasmic reticulum membranes from *Infraspinatus* (IN), *Biceps brachii* (BB). Results are means for muscle X membrane ± SEM.

Muscle	Membrane	
	Mitochondria	Sarcoplasmic reticulum
IN	2.18 ± 0.36	1.79 ± 0.36
BB	2.45 ± 0.36	1.24 ± 0.41

Statistical analysis of the rate constants in Table 3.1 indicated a significant membrane difference but there were no muscle differences. The rate of Mito oxidation was 50% faster than that of SR.

The levels of AA were determined by fatty acid analysis prior to (zero time) and following 60 minutes of oxidation. The amounts of AA at these two time points for membrane systems from each muscle is given in Table 3.2.

Table 3.2. Loss of arachidonic acid during membrane oxidation. Values for initial (zero time) and 60 mins are the mean ± SEM percent AA (of total fatty acids). IN, BB and LD denote muscles *Infraspinatus*, *Biceps brachii* and *Longissimus dorsi.*

	Membrane					
	Sarcoplasmic Reticulum			Mitochondria		
Muscle	Initial	60 mins	% loss	Initial	60 mins	% loss
IN	6.97 ± 0.80	2.39 ± 0.40	66.6 ± 6.0	8.79 ± 0.72	1.60 ± 0.35	80.9 ±5.1
BB	5.95 ±0.93	1.58 ± 0.46	69.3 ± 7.0	7.80 ± 0.72	1.99 ± 0.35	74.8 ±5.1
LD	6.16 ± 1.61	1.20 ± 0.79	80.5 ± 11.4	7.72 ± 0.93	1.75 ± 0.46	77.0 ±6.6

Mito contained significantly higher levels of AA than SR prior to initiation of the oxidation reaction. One hour of oxidation resulted in large losses of AA (up to 80%) and at this time AA levels did not differ significantly between muscles or membrane systems. There was a trend for Mito to show a greater percent loss of AA than SR. This was consistent with the TBARS data (Fig. 3.1 and Table 3.1), thereby supporting the idea that AA in Mito is more susceptible to oxidation than that found in SR

3.4. Molecular species containing arachidonate (AA) in phosphatidylethanolamine (PE) phospholipids of muscle mitochondrial (Mito) and sarcoplasmic reticulum (SR) membranes.

The results of the membrane oxidation studies (Fig. 3.1) provided evidence that Mito lipids are more susceptible to oxidation than SR lipids. Furthermore, fatty acid analysis (Table 3.2) indicated that most of the AA was oxidized within 60 minutes of initiating the oxidation reaction.

Our earlier work (Section 2.7, Table 2.7) had shown that PE was the major reservoir of AA in Mito and SR, in all muscle investigated. The oxidation studies were carried out in liposomes containing the same amounts of lipid as seen in each native membrane system. Therefore the results from Fig. 3.1, Tables 3.1 and 3.2 suggest that that AA in Mito is in a form that is more susceptible to oxidation than that occurring in SR PE.

Muscle phospholipids, especially those of the PE class, differ from most other tissues in that they contain high levels of plasmalogens, a unique subclass of phospholipids characterised by the presence of a vinyl ether substituent at the 1-position of the glycerol backbone to which the fatty moiety is linked. In diester phospholipids the fatty acids are ester-linked at the 1-position. Both plasmalogens and diester-phospholipids have the fatty acid at position-2 ester-linked. These structural differences between the two phospholipid types are illustrated in Figure 3.2.

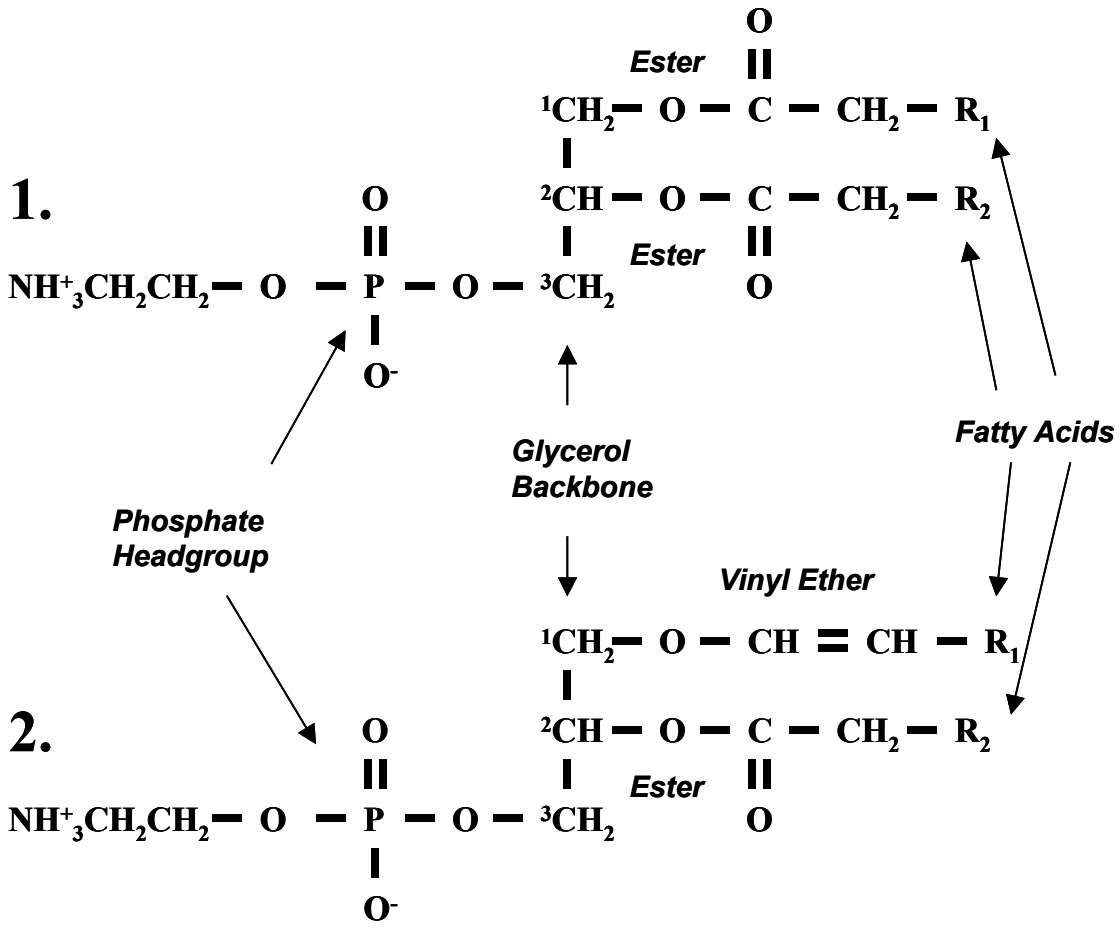

Figure 3.2. Structural elements diester (1) and plasmalogen (2) phospholipids.

Therefore, the higher oxidative susceptibility of Mito could be postulated to be due to a higher ratio of diester-linked AA to plasmalogen AA in this membrane system compared to that of SR. To test this hypothesis, the isolated Mito and SR PE classes from the three muscles (IN, BB and LD) were subjected to a HPLC methodology capable to separating diester-phospholipids from plasmalogens. HPLC traces indicating AA species are shown in Figure 3.3.

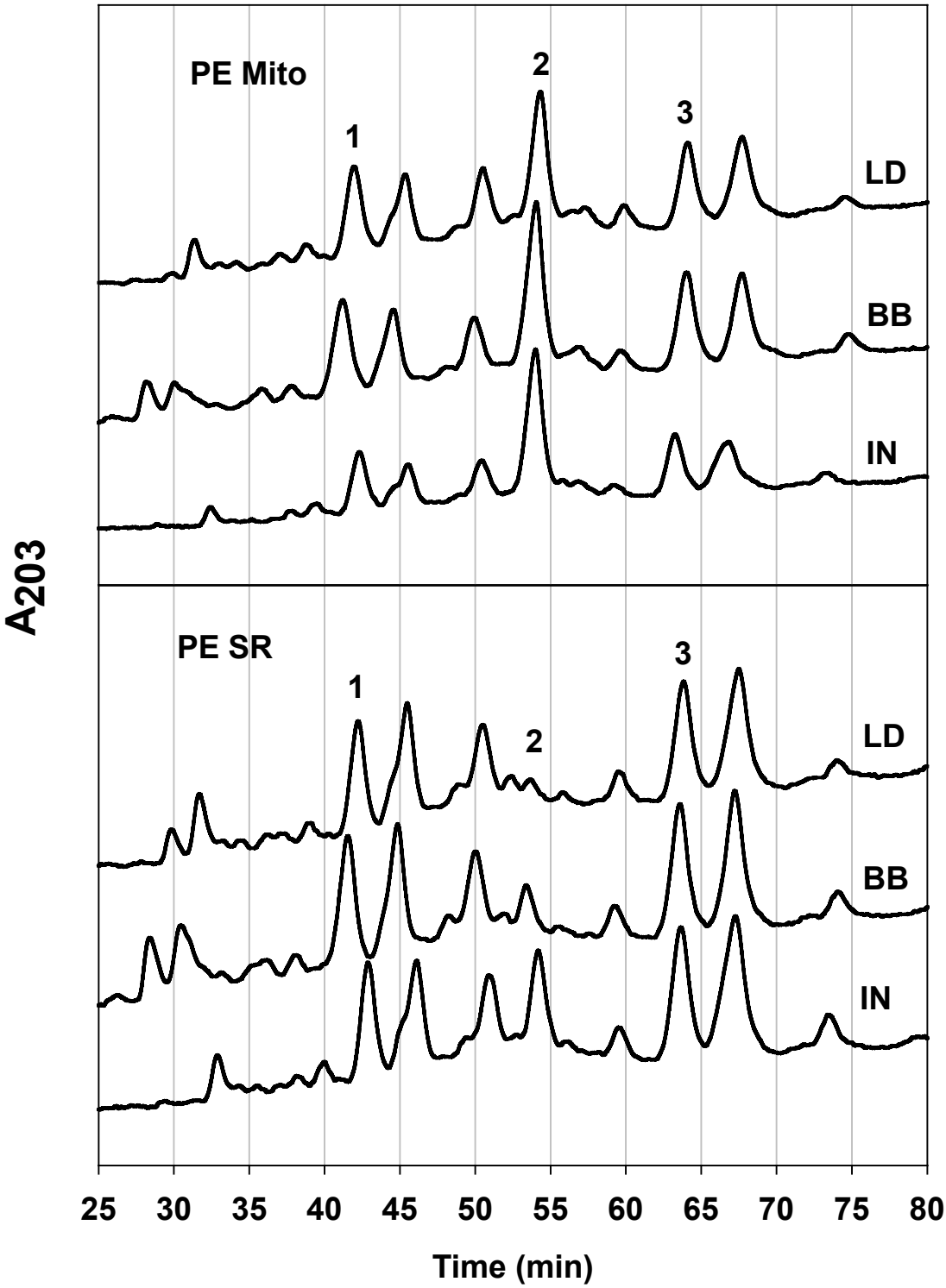

Figure 3.3 HPLC traces of mitochondrial (Mito) and sarcoplasmic reticulum (SR) phosphotidylethanolamine (PE) classes isolated from *Infraspinatus* (IN), *Biceps brachii* (BB) and *Longissimus dorsi* (LD) bovine muscles. Peaks 1, 2 and 3 identify individual arachidonic acid molecular species of 16:0 plasmalogen, 18:0 diester and 18:0 plasmalogen, respectively.

The most striking feature of the chromatograms in Fig. 3.3 is the higher abundance of peak 2 in Mito PE compared to that found in SR PE. Based on its retention time, Peak 2 was tentatively identified as the diester molecular species of composition 18:0;20:4 i.e., 18:0 at position-1 and 20:4(AA) at position-2. Fatty acid analysis of peak 2 confirmed this identification. Quantitative values (as mole percent) of diester molecular species 18:0:20:4 (peak 2) is presented in Table 3.3

Table 3.3 Mole percent of diester molecular species 18:0;20:4, corresponding to peak 2 of traces in Figure 3. IN, BB and LD denote muscles *Infraspinatus*, *Biceps brachii* and *Longissimus dorsi.*

Muscle	Membrane	
	Mitochondria	Sarcoplasmic reticulum
IN	10.84	4.74
BB	10.03	1.69
LD	9.34	0.50

Based on their retention times, peaks 1 and 3 were found to contain AA plasmalogen species. Peak 1 contained the 16:0(P);20:4 species whereas peak 3 contained 18:0(P);20:4 (here P is used to indicate a plasmalogen species). However, while fatty acid analysis of these peaks confirmed the presence of these two plasmalogen species in peaks 1and 3, it also indicated the presence of diester 16:0;20:3 and 18:0;20:3 in these two peaks. Diester 16:0;20:3 and 18:0;20:3 have the same effective chain lengths as plasmalogen species 16:0(P);20:4 and 18:0(P);20:4, respectively, and therefore can not be resolved from one another by the HPLC method employed here.

Due to the presence of two molecular species in peaks 1 and 3, AA plasmalogen species could not be quantitated. However, from total fatty analysis of the PE phospholipid class (see Table 2.5, in Section 2) a percent distribution of diester and plasmalogen AA of total AA could be estimated and these results are presented in Table 3.4.

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Table 3.4. Percent distribution of phosphotidylethanolamime (PE) arachidonic acid (AA, 20:4) as diester or plasmalogen species. IN, BB and LD denote muscles *Infraspinatus, Biceps brachii* and *Longissimus dorsi.*

Muscle	Membrane			
	Mitochondria		Sarcoplasmic reticulum	
	diester	plasmalogen	diester	plasmalogen
IN	39.6	60.4	19.8	80.2
BB	41.6	58.4	7.5	92.5
LD	40.1	59.9	2.4	97.6

In Mito approximately 40% of AA molecular species were of the diester form and this amount did not differ significantly between muscles. On the other hand, in SR percent of AA in the diester form displayed large muscle differences with LD having the lowest levels and IN the highest with BB falling intermediate between these two. Regardless of muscle type, SR always displayed a lower percentage of diester AA species.

The results of Fig.3.3 and Tables 3.3 and 3.4 clearly show that a large proportion AA in Mito is present in the diester form and that this proportion is relatively constant between muscles. While SR always has less AA in the diester form than Mito, pronounced muscle differences are evident. These findings provide strong support for the hypothesis that Mito phospholipids are more prone to oxidation than their SR counterparts due to lower levels of AA in the oxidative-resistant plasmalogen than that found in SR.

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MEAT & LIVESTOCK AUSTRALIA

4. Ribose treatment of beef: An investigation of processing, cooking and storage conditions of ribose-treated meat on warmed-over flavour

4.1. Background

There are antioxidants available that can effectively control warmed-over flavour (WOF) in pre-cooked meat products (Bailey, 1988) but there are shortcomings with their use. Firstly, the safety of synthetic antioxidants will always remain under a cloud of government and public safety debate. Secondly, the use of "natural" or "organic" antioxidant compounds can produce adverse flavour interactions in meat products because many of these compounds are crude extracts sourced from tea, olive and grape seeds. More alarming is the observation by Japanese researchers (Kato *et al.,* 2000) that polyphenols, the active antioxidant in these extracts, can increase the mutagenicity of cooked beef when included in the ground raw beef. In view of these considerations, we decided to investigate the control of WOF development through the generation of *in situ* antioxidants that are produced naturally during the cooking process.

It has been reported, by Danish researchers, that when meat was cooked to internal temperatures in the range 60°C-70°C, and subsequently chill stored, increases in WOF development correlated with increasing internal meat temperature (Mielche, 1995; Mielche and Bertelsen, 1993, 1995). They also observed that as the internal meat temperature increased from 70°C to 100°C, WOF during chilled storage remained constant. They proposed that heating accelerated the oxidation of phospholipids through the release of protein-bound iron. The free iron released during the cooking process was proposed to be catalytically active in initiating lipid oxidation. At very high internal meat temperatures (> 100°C) they observed a very remarkable increase in the oxidative lipid stability of the meat. Indeed, they found meat cooked in this manner was oxidatively stable in the presence of air for 7 days at 4°C.

Cooking meat to internal temperatures of >100°C causes a marked increase in browning products. These browning products are known as Maillard reaction products and have been shown to act as effective antioxidants (Bailey, 1988; Bailey *et al.,* 1997). Unfortunately, in order to achieve internal meat temperatures of >100°C the meat has to be autoclaved or retorted. This type of processing, while improving the oxidative stability of the meat lipids, completely destroys desirable texture and produces an unpleasant burnt taste in the meat.

Based on this background information from other researchers we hoped to be able to develop novel treatments for meat and meat products that would result

in sufficient Maillard browning to produce "natural" antioxidant properties without adversely impacting on meat flavour and texture.

Maillard browning products result from the reaction between reducing sugars and amino acids. Meat contains low levels of free reducing sugars such as ribose and glucose. During the cooking process these reducing sugars react with the amino acid lysine in proteins to produce Maillard reaction products that impact on meat flavour and aroma.

It has recently been shown that very effective Maillard antioxidants can be produced without excessive browning occurring (Morales and Jimenez-Perez, 2001). Based on these findings, our idea was to increase the level of such Maillard antioxidants by the addition of reducing sugars to the meat. We proposed to inject meat with the required sugar and following cooking, determine the effects of such treatments on development of lipid oxidation, determined as TBARS, during subsequent refrigerated storage of the product. We chose to examine the effects of the reducing sugars glucose, fructose and ribose since these three sugars had been shown to produce non-toxic antioxidant Maillard products when heated with proteins (Kato *et al.,* 2000; Jing and Kitts, 2002).

4.2. Experimental

Meat

Forequarter blade (*Triceps brachii*) was the cut employed in these experiments since earlier work in this project had shown that this muscle is prone to WOF development and its choice is also in line with the project rationale of value-adding to lower quality meat cuts.

Whole blades were obtained from Brady's Belmont Butchery (Tingalpa, Qld). All meat was MSA Grade 3 and full traceability back to property of origin was obtained for each cut. The blade was trimmed of fat and connective tissue in the laboratory, and all muscles other than the *Triceps brachii* (TB) were removed. The TB was cut into four portions, each of approximately 300 grams, and each portion was randomly assigned to control (water), glucose, fructose or ribose injection.

Sugar injection

The three TB portions assigned to sugar injection were weighed and then injected with a 6.4% solution of the required sugar (glucose, fructose or ribose) in demineralised water. The meat was injected at different sites (usually 4-6) to a depth of 2 cm using a multi-needle injector (25 18G needles arranged 5X5 in square with a side of 1.5 cm) until the weight of the portion increased by 5%. This resulted in a final sugar concentration of 0.4% (grams of sugar per 100 ml of muscle water, assuming muscle contains 80% water). Between each injection the portion was blotted dry with paper towels in order

to remove excess sugar solution from the meat surface. The control TB portion was treated in an identical manner except that demineralised water was injected.

Cooking and storage

The treated portions were cooked in a fan-forced convection oven at 200°C for 25 minutes. At the end of the cooking period the centre of the portions reached 76°C and the meat was then removed from the oven and rested for 10 minutes prior to being cut into approximately 3 mm thick slices. The slices were randomly assigned to 4 storage regimes of 1, 2, 3, and 6 days and placed in unsealed plastic bags and stored at 5°C for the required time. As a zero time point some of the slices were vacuumed sealed in plastic bags and immediately stored at -70°C.

Chemical analysis of chill stored meat samples

At the end of each storage period the corresponding slices were rewarmed at 75°C in a water bath for 10 minutes. The slices were then finely chopped in a food processor and weighed amounts were extracted and assayed for lipid oxidation products by the TBARS assay.

4.3. Investigation of the effect of reducing sugars on TBARS

The results for the effects of the three sugars on the TBARS value of rewarmed chill-stored beef slices are shown in Figure 4.1.

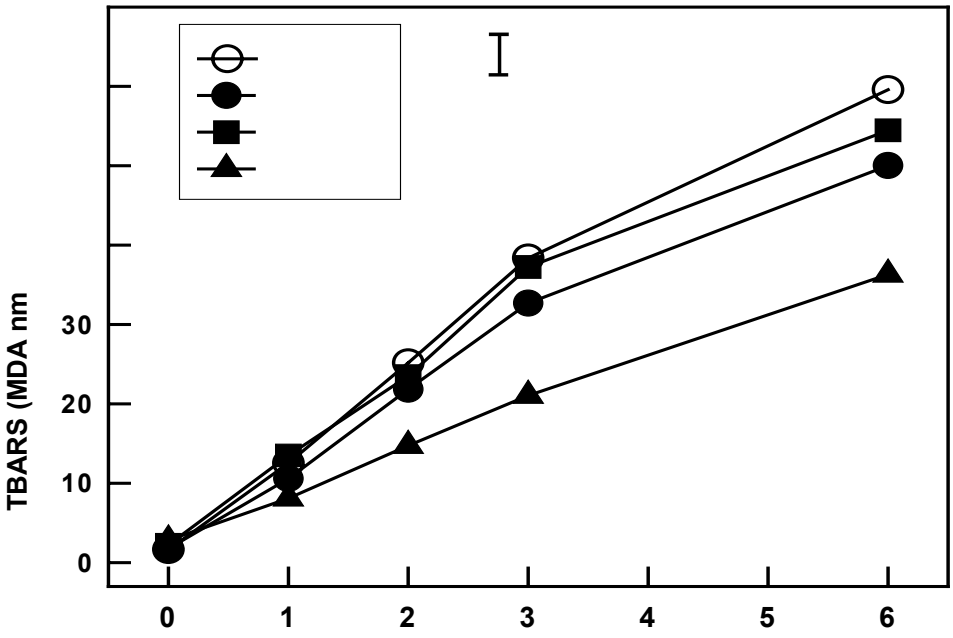

Figure 4.1. The effect of reducing sugars on TBARS values in rewarmed chill-stored beef slices. The results are the means of three replicate experiments.

The development of lipid rancidity, determined as TBARS, in the control blade increased linearly with increasing storage time and was very similar to that observed in our earlier trial (see Section 1, Fig. 1.3). The glucose and fructose treatments tended to give slightly lower TBARS values than the control but these differences were not statistically significant.

The ribose treatment, on the other hand, resulted in a significant lowering of TBARS to 40% of the control value at 1, 2, 3, and 6 days storage. Based on these results, it was decided to only use ribose in subsequent investigations.

4.4. Effect of ribose concentration on TBARS

Having established that ribose was the most effective reducing sugar of the three tested sugars, it was of interest to determine the relationship between ribose dosage in meat and the reduction in TBARS produced. For this experiment six concentrations of ribose (0, 0.1, 0.2, 0.4, 0.6 and 0.8%) were examined and their percent TBARS relative to a control (no ribose) was determined. The results of this experiment are shown in Figure 4.2.

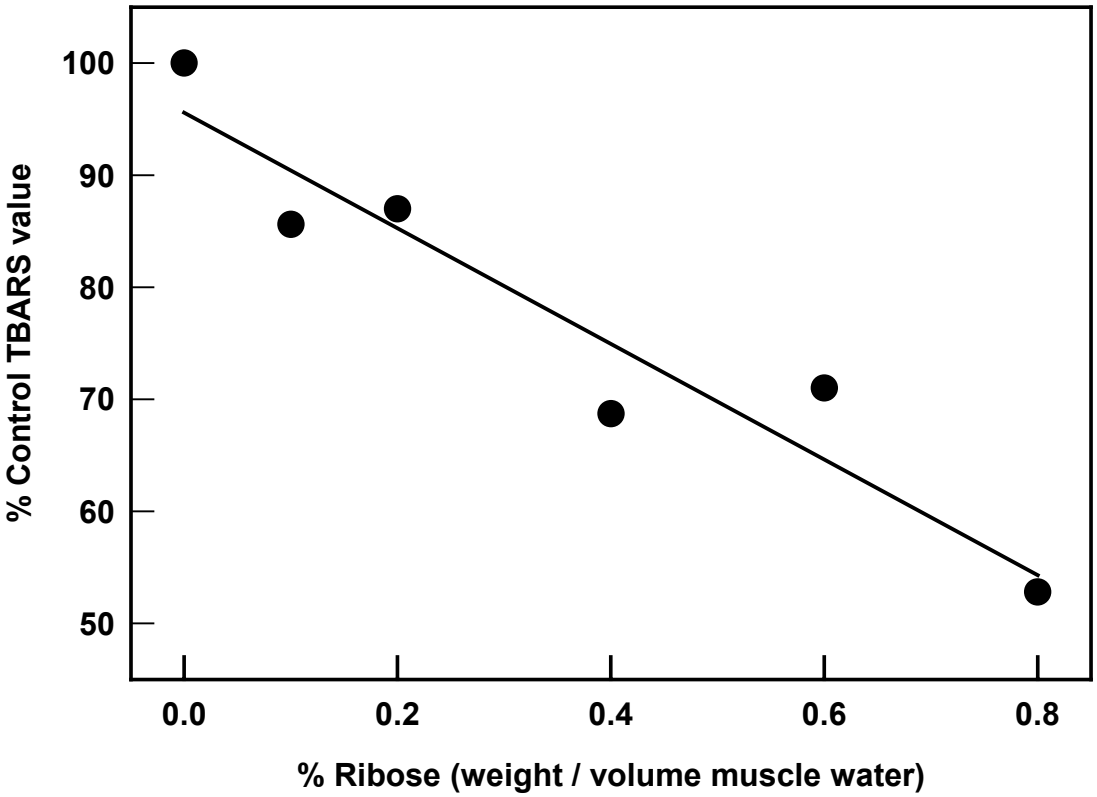

Figure 4.2. The effect of ribose concentration on TBARS in rewarmed chill-stored beef. Each point represents the pooled average of the 1, 2, 3 and 6 day chilled storage samples. The data was obtained from four separate experiments.

The reduction in TBARS produced by ribose treatment was linearly correlated with ribose concentration. The higher the level of ribose injected into the meat the greater the reduction in TBARS.

4.5. Effect of internal meat temperatures on TBARS in ribose-treated beef

The results of the previous two experiments were obtained from oven-roasted samples, with the oven set at 200°C and the meat cooked to an internal temperature of 76°C. Under these conditions large temperature gradients in the meat are unavoidable and largely uncontrollable. Furthermore, the roasts developed a browned crust indicative of extensive Malliard reaction on the meat surface.

Therefore our results of improved oxidative stability in ribose-treated meat may have been due solely to high temperatures at the meat surface where production of Maillard browning products was greatest. In order to test this hypothesis it was decided to investigate the effects a non-browning cooking regime on subsequent TBARS development in ribose-treated meat. For this purpose the injected meat samples were cooked in a water bath set 1°C higher than the desired final internal meat temperature. The meat was removed from the water bath when the desired temperature was reached at the geometric centre of the meat cut. This method of cooking effectively prevented surface browning of the meat.

Four internal meat temperatures, 75°C, 80°C, 85°C and 90°C, were investigated and at each of these temperatures a control and ribose injected (0.4 % weight of muscle water) sample were examined. The results of this experiment are shown in Figure 4.3.

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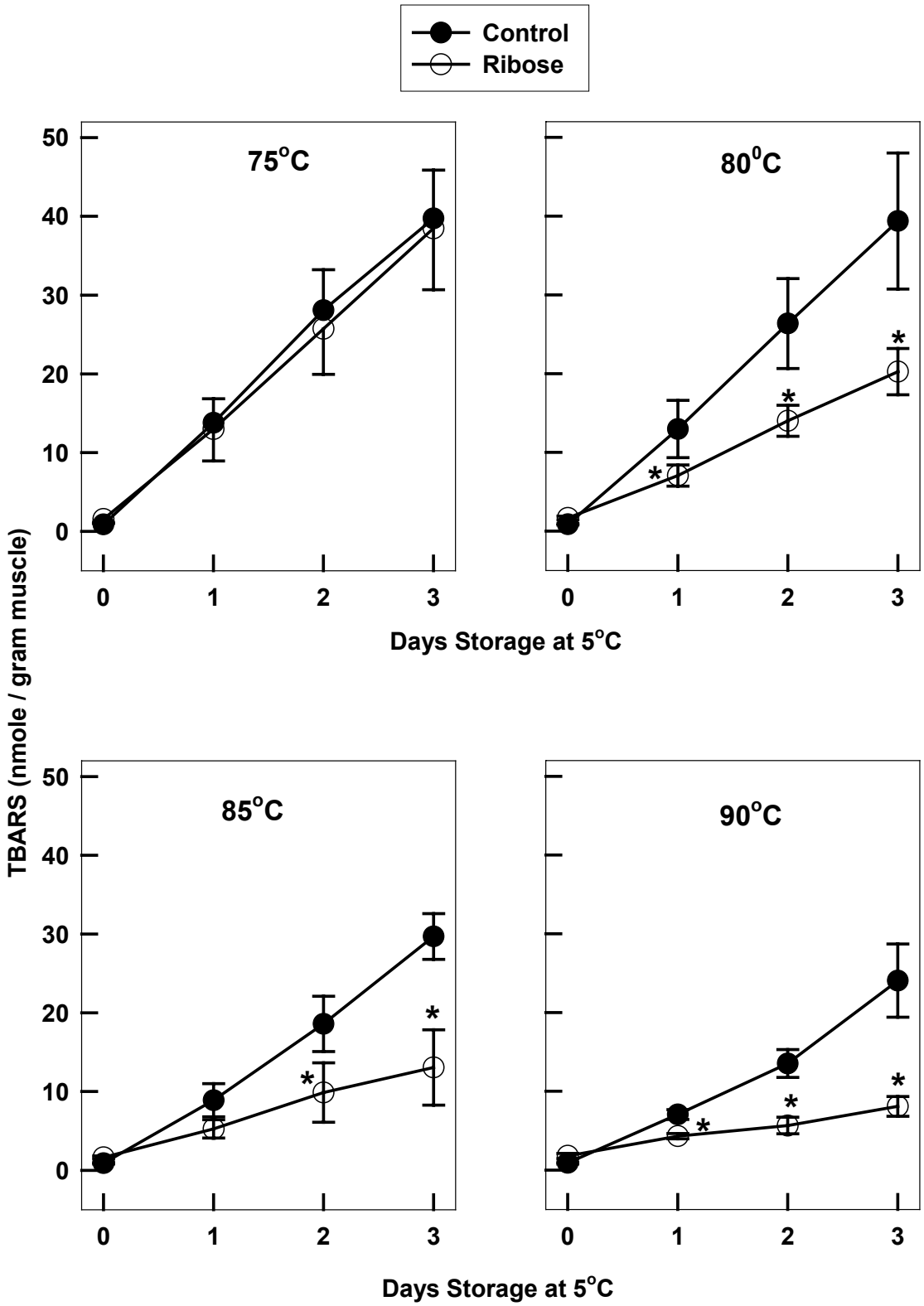

Figure 4.3. Effect of internal meat temperature during non-brown cooking in control and ribose treatment blade on TBARS formation during chill stored cooked beef. Results are the means ± standard deviations of four experiments.

Development of lipid rancidity, determined as TBARS values, during 3 days chilled storage did not differ between control or ribose-treated when meat was cooked to an internal temperature of 75°C in a water bath set at 76°C.

In meat cooked to an internal temperature of 80°C, the ribose-treated samples displayed significantly lower TBARS at each of the storage time points. Overall at this temperature, an approximate 47% lowering of TBARS to that of the controls was observed at each of these time points. Rate of TBARS increase in the control samples at 80°C was similar to those heated to 75°C.

At 85°C it was observed that the TBARS in the control samples were lower at each storage time point than their counterparts cooked to internal temperatures of 75°C or 80°C. However, at 85°C the ribose-treated meat still displayed lower TBARS than the controls. The 2 and 3 day samples displaying significantly lower values than the control at these time points.

At 90°C, TBARS in the control samples were lower than corresponding samples at 85°C. At each of the storage time points the ribose-treated samples had significantly lowers TBARS values than the corresponding control samples.

Cooking of ribose-treated meat to 75°C, in a 76°C water bath, resulted in samples cooked to the same internal temperature as obtained in the oven-roasted experiments but without development of a browned crust. Also, with the former cooking protocol a 1°C temperature differential was maintained between the outside of the meat and its geometric centre whereas in the latter protocol this temperature differential was 124°C (200°C minus 76°C). With non-browning water bath cooking to an internal temperature of 75°C, it was observed that the ribose treatment had no significant effects on TBARS values during subsequent chilled storage (Fig. 4.3, top left panel). Therefore, the reduction of TBARS seen in the oven-roasted experiment (Fig. 4.1) could have been due to either the antioxidant effects of the brown crust or internal meat temperatures greater than 75°C.

Increased oxidative stability during chilled storage of ribose-treated meat becomes evident at internal meat temperature of ≥ 80°C (Fig. 4.3, top right hand panel). Therefore, it can be concluded that surface browning of the meat is not required to obtain oxidative stability in ribose-treated meat.

In the case of oven roasted ribose-treated meat it is not possible to distinguish between the antioxidant effects produced by browning from those produced by internal temperatures of ≥ 80°C. However, the fact that similar

reductions in TBARS values in ribose-treated samples, relative to controls, are seen in oven roasted (Fig. 4.1) versus 80°C water bath cooked samples (Fig.

4.3, top right hand panel) suggest that internal temperatures averaging close to 80°C could entirely account for the observed oxidative stability in the oven roasted samples.

The results of this experiment also uncovered another effect, apart from ribose treatment, that resulted in oxidative stability in cooked beef during chilled storage. This effect was internal meat temperatures of ≥ 80°C. The relationships between internal cooking temperature and the subsequent oxidative stability (TBARS value) of control and ribose-treated meat are more clearly seen in mesh plot presented in Figure 4.4.

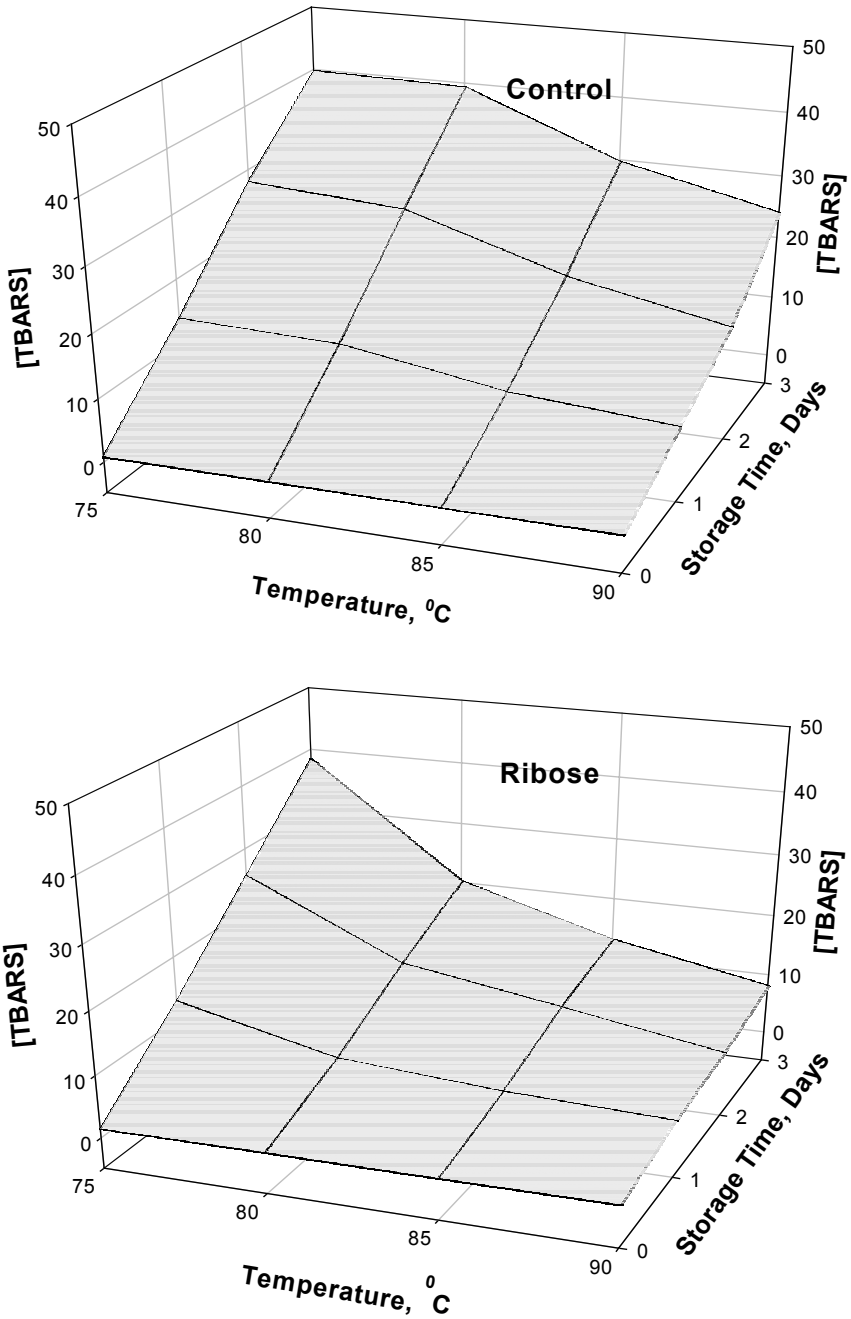

Figure 4.4. Mesh plot showing relationships between internal temperature of cooked meat and chilled storage time on TBARS development control and ribose-treated cooked beef (upper and lower panels, respectively). Data points used in the plots were the means from four replicate experiments.

The data presented in Figs. 4.3 and 4.4 indicated that at each internal cooking temperature TBARS increased linearly during subsequent chilled storage time. Linear regression of the data allowed the rates of oxidation (i.e. TBARS/day) for each treatment and temperature combination to be determined. These rates are presented in Table 1.

Table 4.1. Rates of lipid oxidation (TBARS/Day) in control and ribose-treated beef cooked to internal temperatures of 75°C-90°C and subsequently stored at 5°C. Rates were calculated from the slopes of the lines in Figure 4.3 obtained from linear regression.

Internal meat temperature	Oxidation Rate (TBARS/Day)	
	Control	Ribose
75°C	13.51	12.84
80°C	13.13	6.85
85°C	9.65	4.59
90°C	7.59	2.85

4.6. Sensory evaluation of warmed-over flavour in ribose-treated beef

Our previous sensory study and its correlation to TBARS (Section 1, Fig. 1.7) indicated that half maximal sensory response to warmed-over aroma and flavour occur at a TBARS value of 4.08. If TBARS exceed this value when chilled beef is rewarmed it becomes detectable as warmed-over flavour by the sensory panellists. Examination of the mesh plot for cooking temperature and chilled storage time (Fig. 4.4) indicated that only in ribose-treated beef cooked to internal temperatures of 85°C or 90°C would the TBARS value remain low enough to avoid sensory detection as warmed-over aroma and flavour.

Intuitively, it was felt that a sensory evaluation beef cooked to 90°C could be compromised by dry meat caused by high cooking losses at this temperature. In order to substantiate this belief, the effect of ribose treatment on the moisture content of cooked beef was analysed. Moistures determined on the water bath cooked samples were plotted against their corresponding internal meat temperatures and these results are presented in Figure 4.5.

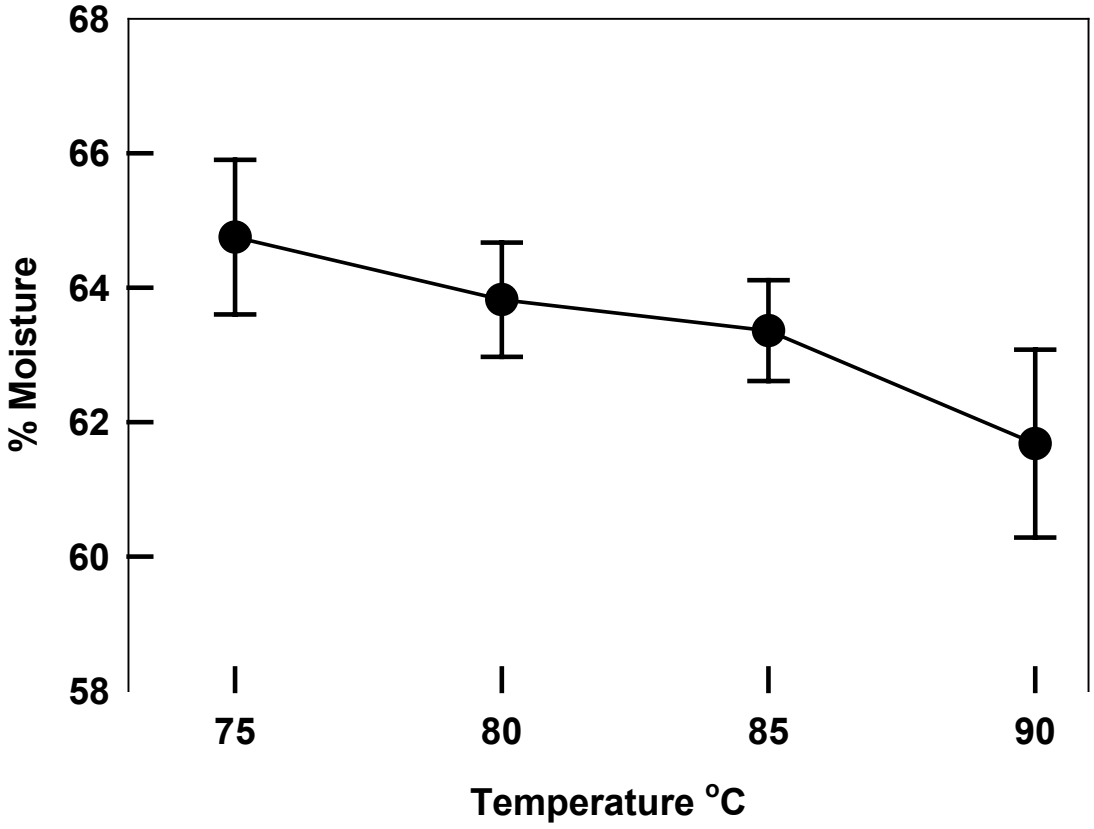

Figure 4.5. The moisture content of cooked beef as a function of internal meat temperature of control and ribose-treated beef. Results are presented as means ± standard deviations. The results of three replicate experiments are presented.

Based on moisture content, ribose treatment was observed not to effect cooking loss and so the results presented in Fig. 4.5 are the pooled means for control and ribose-treated samples. As can be seen in Fig. 4.5, the moisture content decreased with increasing internal meat temperature. The moisture contents of meat cooked to 80°C and 85°C did not significantly differ from one another. Moisture content at these two temperatures was 1.8% less than that at 75°C but was 5% higher than that at 90°C.

Based on the results from Figs. 4.4 and 4.5 it was decided to cook the meat to an internal temperature of 85°C for the sensory evaluation of a 0.4% ribose treatment on warmed-over flavour. The design of the sensory evaluation was the same as that used previously (see Report on Milestones 3, 4 and 5) except that the meat was cooked to an internal temperature of 85°C in a water bath set at 86°C. Sensory scores for the meat attributes of aroma, flavour, tenderness, juiciness and overall quality are shown in Figure 4.6.

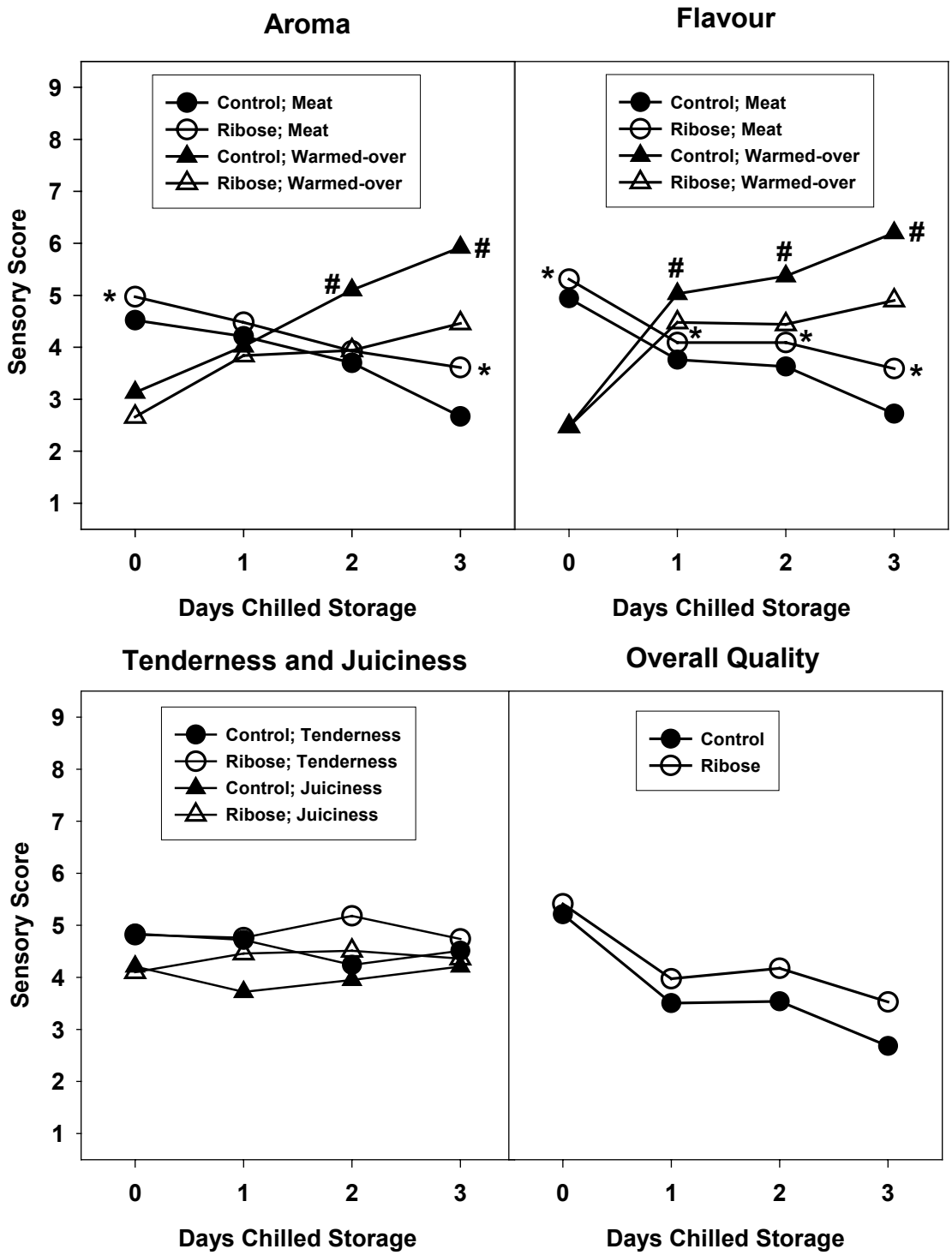

Figure 4.6. Average sensory scores for meat and warmed-over aroma (top left hand panel), meat and warmed-over flavour (top right hand panel), tenderness and juiciness (bottom left hand panel) and overall quality (bottom right hand panel). The * and # symbols are used for meat aroma / flavour and warmed-over aroma / flavour attributes, respectively and indicate a significant difference between control and treatment means of these attributes at the same time point.

Meat and warmed-over aroma
The average score given for meat aroma declined with increasing storage time in both control and ribose-treated samples. Interestingly, the panellists scored the ribose-treated samples higher for meat aroma than the controls but these differences reached significance only in the zero (frozen) and 3 day samples. Conversely, the average scores for warmed-over aroma increased with increasing storage time in both the control and ribose-treated samples. At 2 and 3 days the scores for warmed-over aroma were significantly lower for the ribose-treated meat compared to the control samples. Overall, the loss of meat aroma during chilled storage was paralleled by the development of warmed-over aroma. Ribose treatment reduced the development of warmed-over aroma while enhancing meat aroma.

Meat and warmed-over flavour
The average sensory score for meat flavour declined with increasing storage time. However, at each time point the panellists scored the ribose-treated samples significantly higher for meat flavour. The average scores for warmed-over flavour increased with storage time but at each time point the scores for warmed-over flavour in the ribose-treated samples were significantly less than those given to the control samples.

Tenderness and juiciness
The panellists detected no significant difference in tenderness between the two treatments and tenderness did not change significantly with storage time. The average score for juiciness also did not change significantly with storage time. However, the panellist's scored the ribose-treated samples juicier, on average, than the controls and this difference was significant.

Overall quality
The average score given by the panellists for overall quality declined with increasing storage time but at each time point the ribose-treated samples received a significantly higher score than the controls.

4.7. Relationship between warmed-over aroma and flavour sensory scores and TBARS

In our previous sensory evaluation of warmed-over flavour (Section 1, page 28), we found that warmed-over aroma and flavour sensory scores and their corresponding TBARS value gave an excellent fit to curves calculated using the Hill equation. Data from the present sensory study were also modelled using this equation, and as seen from the Hill plots in Figure 4.7, the current experimental data also gave an excellent fit to the curves calculated from this equation.

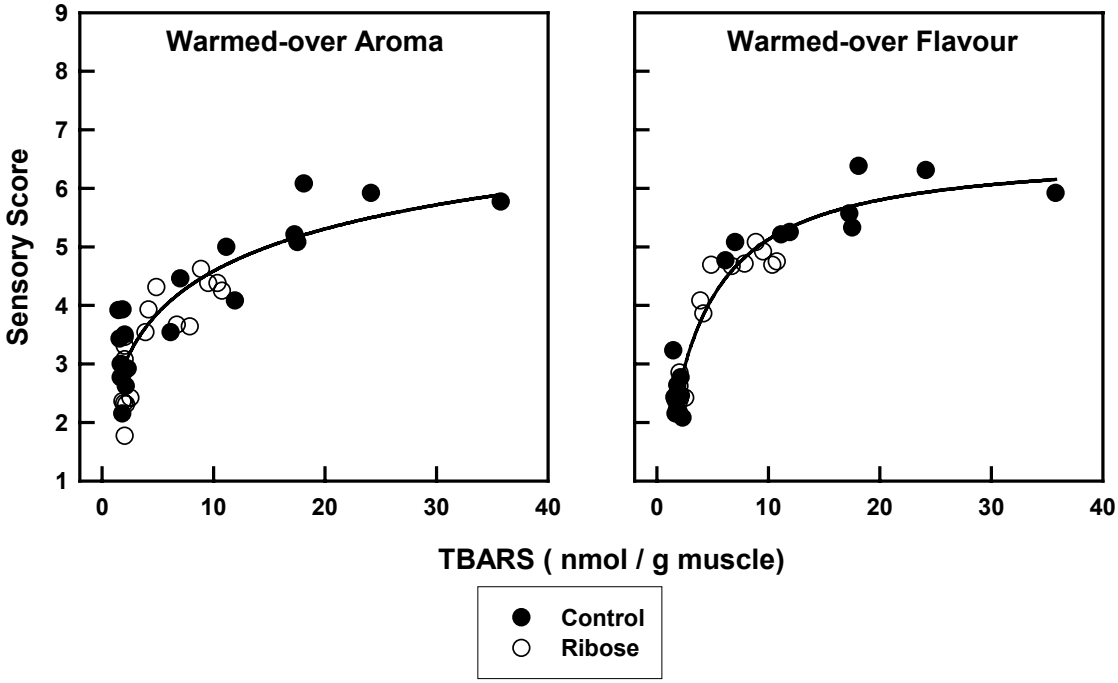

Figure 4.7 . Average sensory scores for warmed-over aroma (left panel) and flavour (right panel) plotted against their corresponding TBARS value. The fitted curve was calculated using the Hill equation.

The fitted Hill plot gave a correlation coefficient of 0.97 for sensory scores of warmed-over flavour with their corresponding TBARS value. The model predicted that a maximum sensory score of 6.6 would be attained and that half this score (i.e. 3.3) would be given for samples with a TBARS value of 3.0.

4.8. Conclusions and development of a predictive model for warmed-over flavour development.

Efficacy of ribose in TBARS reduction

Fig. 4.1 showed that ribose when added to raw meat was the only reducing sugar, of three tested, that would subsequently reduce lipid rancidity in beef during chilled storage. Canadian work (Jing and Kitts, 2002), studying Maillard reaction products in a model system of reducing sugars and casein as protein, found that ribose reacted faster with casein than glucose or fructose. Glucose and fructose are aldohexoses (6 carbon sugars) while ribose is aldopentose (5 carbon sugar) and generally pentoses are more reactive than hexoses in their reaction with the basic amino acids in proteins.

Interestingly, the Canadian study also found that the ribose-casein reaction products had antioxidant properties while those from glucose and fructose

displayed none. Furthermore they reported that none of the reaction products from any of the sugars were toxic when tested in a cultured cell line.

When we tested the dose response of ribose on reduction of lipid oxidation, determined as TBARS, a linear response was observed (Fig. 4.2). We decided on a level of 0.4% (weight per 100 ml of muscle water, assuming meat to be 80% water) for sensory work for two reasons. Firstly, at this concentration the ribose was effective in TBARS reduction (Figs. 4.1 and 4.2). Secondly, Japanese research (Kato *et al.,* 2000) had shown that at this concentration ribose produced no mutagenic compounds when added to ground beef and subsequently cooked at 200°C for 20 minutes.

Using an oven temperature of 200°C to cook ribose-treated meat to an internal temperature of 76°C resulted in extensive browning of the meat surface and a large temperature gradient (124°C) from the surface to the centre of the meat. This made it difficult to determine if the resulting reduction in TBARS in the treated samples resulted from the browning Maillard products produced by higher temperatures at the meat surface or other stages of the Maillard reaction occurring at lower temperature within the meat. A Spanish study (Morales and Jimenez-Perez, 2001) on Maillard reaction of reducing sugars and free amino acids concluded that browning was not directly related to antioxidant properties.

When ribose-treated meat was cooked in a water bath whose temperature was set 1° higher than the desired internal meat temperature, no browning occurred. Under these conditions reduction of TBARS was only evident at temperatures ≥ 80°C (Fig. 4.3). These results suggest that TBARS reduction in oven roasted ribose-treated meat may not have been due to browning but rather by temperatures ≥ 80°C, reached internally in the meat during cooking.

Contrary to the findings of Danish workers (Mielche and Bertelsen, 1995) we observed that untreated meat cooked to internal temperatures of 75°C-90°C resulted in a reduced oxidation rate (TBARS/Day) during subsequent chilled storage (Fig. 4.4). The rate of TBARS formation at 90°C was 56% and 22% of that seen at 75°C in control and ribose-treated meat, respectively (Table 4.1).

Sensory evaluation of ribose-treated beef

Ribose-treated meat was cooked to 85°C and chill stored for 0, 1, 2, and 3 days before being rewarmed and served to sensory panellists familiar with warmed-over flavour. From the mean sensory scores for meat aroma, warmed-over aroma, meat flavour, warmed-over flavour, tenderness, juiciness and overall quality, ribose-treatment was shown to increase meat aroma, meat flavour, juiciness and overall quality. Ribose treatment also significantly reduced warmed-over aroma and flavour in chill stored beef.

Ribose treatment therefore has three effects in cooked beef. Firstly it enhances beef flavour and aroma and this effect is evident in both fresh and chill stored beef. Ribose is a well known beef flavour precursor (MacLeod and Seyyedain-Ardebili, 1981; Farmer and Hagan, 2002) and so the production of beefy flavours and aromas above that seen in untreated meat is not altogether surprising.

Secondly, the Maillard reaction products of ribose, produced at temperatures ≥80°C, have antioxidant properties that are effective in reducing warmed-over flavour and aroma. We demonstrated that the final Maillard reaction products of brown pigments were not required for the observed antioxidant effects in ribose-treated meat. The first steps in the Maillard reaction involve the addition of the carbonyl group from the reducing sugar to the primary amino group in a protein (Whitfield, 1992). With aldose sugars, such as ribose, the condensation product is converted to a 1-amino-1-deoxy-2-ketose. At elevated temperatures the 1-amino-1-deoxy-2-ketose forms a methyl-2,3-dicarbonyl compound which is in equilibrium with the corresponding reductone. The reductone has potent antioxidant properties. Our observation that the antioxidative property of ribose-treated meat increased with increasing temperature probably reflects greater production of the reductone at the elevated cooking temperatures.

The third effect observed in ribose-treated beef was an increase in sensory score for juiciness. We know from moisture determination on treated and control samples that ribose treatment had no effect on the moisture content (Fig. 4.5). Therefore some effect of the ribose treatment leads to the sensory perception of increased juiciness. It seems unlikely that the reaction products of ribose were responsible for the higher sensory score of this attribute. These compounds are present in meat in the parts per million levels and at such low concentrations would only be expected to impact on flavour and aroma. It seems likely that the majority of the injected ribose remains unreacted in the meat following cooking and the taste panellists perceive the higher level of the free carbohydrate in this meat as increased juiciness.

Relationship between sensory scores and chemically determined values of lipid oxidation (TBARS)

Sensory scores for warmed-over aroma and flavour when plotted against values for chemically determined lipid oxidation, i.e. TBARS, indicated a curvilinear relationship (Fig. 4.7). Our data is in very good agreement with that of Danish researchers (Stapelfeldt *et al.,* 1992) who reported very similar results, also in cooked beef slices. The Danish workers chose a logarithmic model to predict the relationship between sensory scores and TBARS since they observed a reasonable linear relationship between sensory scores and log-transformed TBARS values. We preferred the Hill equation to model the relationship between sensory score and TBARS because we felt that this

equation would account for flavour and aroma saturation due to cooperative binding of the odorant and flavour molecules to olfactory and flavour receptor sites in the noses and mouths of the panellists.

The Hill plot accounted for 93% of the observed variance in the experimental data. Furthermore, the plot allowed the Hill parameters of the TBARS value for half maximal sensory response and maximal sensory score for warmed-over flavour to be determined as 3.0 and 6.6, respectively. In our previous sensory trial the TBARS value producing half maximum sensory response was calculated to be 4.1.

Using the data for rate of TBARS formation (Table 4.1) and the Hill parameters calculated from Fig. 4.7, the chilled shelf life prior to consumer detection of warmed-over flavour for control and ribose-treated beef cooked to internal temperatures of 75°C-90°C is presented below in Table 4.2.

Table 4.2. Prediction of chilled shelf life prior to development of detectable levels of warmed-over flavour in control and ribose-treated beef cooked to internal temperatures of 75°C-90°C.

Internal meat temperature	Chilled shelf life (hours)	
	Control (untreated)	Ribose -treated
75°C	5.3	5.6
80°C	5.5	8.1
85°C	7.5	15.7
90°C	9.5	25.3

In our previous sensory trial it was calculated that warmed-over flavour in chilled beef would be detectable in 5 hours. That meat was oven cooked to an internal temperature of 75°C and the predicted shelf life, in Table 4.2, for water bath cooked meat to the same internal temperature is in good agreement with the previous trial.

In untreated beef, cooking the meat to an internal temperature of 90°C will double the "warmed-over" shelf life compared to that cooked to 75°C. However, this will result in 5% less moisture (Fig. 4.5) and probably result in quality problems due to lack of juiciness in the product.

The shelf life of ribose-treated meat cooked to an internal temperature of 85°C is 15 hours and is triple the shelf life of meat cooked to an internal temperature of 75°C. We know from the sensory results (Fig. 4.6), moisture content is not a problem with ribose-treated meat cooked to this internal temperature, because the panellists rate this product as more juicy than the

untreated control. A 24 hour shelf life for ribose-treated meat can be attained only if it is cooked to an internal temperature of 90°C. It remains to be determined if ribose-treated meat cooked to this temperature is still perceived as juicy by consumers

Commercially, one would like to see a shelf life of 24-48 hours in ribose-treated beef oven cooked to an internal temperature cooked of 75°C. The only way to achieve this would be to increase the reaction rate of antioxidant formation in ribose-treated meat. High-pressure treatment of meat may be a possible way to accomplish this, and commercial high-pressure units are already in use.

Clearly, investigation of technologies capable of producing desirable levels of antioxidant and flavour compounds in ribose-treated beef at lower temperatures than employed in this investigation is warranted. If high pressure heat treatments accomplished this goal then it would also have the additional benefit of tenderising the meat (MacFarlane, 1985), thereby making the process amenable to value-adding of the lower quality beef cuts.

4.9. Reference material

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5. Potential Applications for Ribose-treated beef products.

5.1. Emulsion cured beef products

Costs

Emulsion curing involves cooking the meat emulsion immediately following addition of the curing ingredients. A standard brine solution of the curing ingredients (salt, polyphosphates, sucrose, nitrite and erythorbate) costs $1.30 per kilo to produce (materials plus labour). Addition of the brine to 10% of product weight results in cost of 13 cents per kilo of final product.

In 1995 the price of food-grade ribose was prohibitive at $200 per kilo. Three years later the price dropped to less than $100 per kilo thanks to new production methods. Ribose is currently the fastest growing nutraceutical in the United States and prices of $50 per kilo would not be unrealistic as demand for this sugar continues to grow.

Maximum benefits of ribose on the curing process (see below) occur with 1% ribose in the final product. Therefore a brine solution in which ribose replaces sucrose would cost $6.70 per kilo resulting in a 67 cent cost per kilo of product. This is five times the standard curing cost. However good curing effects can occur at 0.2-0.5% ribose and if product quality is acceptable at these concentrations of ribose then the brine cost per kilo of final product will be approximately twice that of the standard brine solution.

Benefits

In emulsion cured beef products ribose has been shown to enhance colour formation of nitric oxide myoglobin (the pink cured meat colour) whereas sucrose has no effect in this regard. Complete formation of cooked cured meat pigments occurs with 1% ribose in the cured product with good colour formation seen at 0.2-0.5% ribose.

Because ribose is a reducing sugar it displays good nitrite decomposition properties. This means that excess nitrite will be destroyed by the ribose and this role is performed by erythorbate in the standard brine. The role of sucrose in the brine is to reduce the harsh taste of the salt and ribose can also fulfil this function. Therefore, it may be possible for ribose to replace both the sucrose and erythorbate of the standard curing brine solution, further reducing the costs.

The major novel benefit of ribose in cooked emulsion products compared to the standard process would be flavour enhancement through production of beefy flavours and aromas and prevention of lipid rancidity.

Processing
No additional processing would be required with use of ribose brines compared to that of the standard brine solution.

5.2. Whole beef carcases
Costs
Ribose treatment of whole carcasses is feasible through the postexsanguination vascular infusion process developed by MPSC, Inc. This process improves beef value through increased meat yields, colour and tenderness. The infusion solution generally consists of 98.5% water, 1% sugars, 0.2% salt and 0.3% phosphates.

Carcases are infused with the above solution at a rate of 10% live weight. From published data on MPSC treated carcases it can be calculated that the infusion results in a 5% carcase weight gain. Assuming that all the infused electrolytes distribute in muscle, then the sugar content in the muscle would be 0.1%.

For a 500 kg live weight animal the cost of the ribose in the infusion solution would be $25. Our results suggest that addition to 0.4% ribose in meat is required in order to achieve significant effects with this sugar. This would increase the ribose cost to $100 per carcase and such a cost is clearly prohibitive.

5.3. Pre-cooked beef: Cook-in-casing and sous vide products
Cook-in casing
Cook-in-casing beef products are usually used for roasts. The roast is placed into a watertight casing (sauce may also be added to the roast) a vacuum is pulled and the casing is sealed. The beef roast is then slow cooked to internal temperatures of 60-75°C. The cooked product is stored at 2-3°C and has a shelf life of approximately 4 weeks. The product can be reheated in the bag prior to serving or removed from the bag and reheated or further processed.

Ribose injection of the raw beef cuts would be an easy preliminary pre-treatment prior to placing the cut into the casing. Use of ribose at 0.4 % of raw meat weight would increase the costs by 60-70 cents per kilogram of meat.

In the United States major meat processors such as Hormel, IBP, Smithfield, and Burnett & Son have introduced precooked roast beef products. These products retail for $12-$18 per kilogram which represents a substantial value-adding for the beef cuts involved.

In view of the value-added nature of these products the 4-5% increase per kilogram cost due to ribose treatment of the raw meat can be easily absorbed.

Hopefully the value of a ribose treated product will be increased if beef flavour is enhanced and off flavour development prevented.

The only special requirement for a ribose treated product is higher internal cooking temperatures (≥ 80°C) needed to produce the desired effects of flavour enhancement and oxidative stability. While the higher processing temperature will incur increased energy costs, these may be offset by the increased shelf-life of the ribose product.

Sous vide

Sous vide is a French term that means under vacuum. Although similar to cook-in casing processing, sous vide technology is more elaborate. The process generally uses meat portions as opposed to roasts. The surface of the meat can be sheared and then sauces are added. Other ingredients may be partially cooked or processed. The meat portions are packaged into preformed pouches or trays which are sealed under vacuum.

The products are cooked to very well defined temperatures and times in order to effect pasteurisation. Following cooking the pouches are rapidly chilled and stored at 2-3°C. As with the cook-in-casing roasts, the sous vide products are reheated prior to serving.

Ribose pre-treatment beef portions would be ideally suited to sous vide products. This is because many of the meat products used in sous vide are currently injected and/or tumbled prior to cooking. Again the value-added nature of the product makes it easier to absorb the additional costs of the ribose.

Warmed-over flavour has been shown to increase rapidly in sous vide beef after 14 days of chilled storage. Ribose treatment of the raw product has the potential to increase this shelf life by delaying the onset of the oxidative processes that lead to WOF development. Increasing the cooking temperatures to ≥80°C should present no major problems because with the sous vide process, as the temperature is increased the time held at that temperature can be reduced as long as pasteurisation is still achieved.

5.4. Ribose and ultra high pressure (UHP) as an emerging technology for pre-cooked beef products.

UHP treatment of meat appeared in the 1970's with the works of MacFarlane reporting improved tenderness in UHP treated meat. Since then renewed interest in UHP food processing has emerged because of its proven ability to inactivate microorganisms. Alongside UHP, moderate temperatures such as 40-60°C, will sterilise more effectively than UHP alone. This combination of heat and pressure may prove to be the most efficient means of destroying

bacterial spores. Therefore UHP plus heat treatment can not only tenderise the meat but also effectively pasteurise it as well.

The introduction of moderate temperatures into UHP processing can affect the Maillard reaction which is responsible for flavour and colour development in cooked foods. Recent research into this area using model systems of sugars and amino acids indicates that UHP accelerates the initial reactions but retards the later reactions involved in colour development.

Research on this project (Section 4) showed that early Maillard reaction products involving ribose were responsible for the observed antioxidant properties. Since UHP has been shown to accelerate these early reactions in model systems, it seems plausible that UHP plus heat could increase the antioxidant efficacy of ribose reaction products. If the results we obtained at 90°C could be improved upon by UHP plus 60°C heating then WOF would be effectively eliminated.

UHP plus heat treatment of ribose-treated meat therefore has the potential to increase the value of pre-cooked beef products above that using current technology. The increased value of the products would result from improved tenderness, longer shelf life for flavour and increased microbiological safety.

5.5. Reference Material

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